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THE PATHOLOGY OF PSITTACOSIS

Discussion with Case Report*

ALVIN G. FOORD

From the Laboratories of the Pasadena Hospital, Pasadena, California

From April 1931 to April 1933 fifty-seven cases of proved psittacosis have occurred in California, forty-seven cases in other states of the union, and during the years 1929 to 1933 approximately eight hundred cases with a mortality rate of 35 to 40 per cent occurred throughout the world (Meyer[7]). Furthermore recent studies, especially those of Meyer and by Hasseltine[2] have shown that birds of the parrot family commonly harbor the virus of the disease and spread the condition to other birds and possibly to man, without showing any symptoms of the disease themselves. Consequently psittacosis must be considered as a possible menace in localities where parrakeets or parrots are raised or sold, and in the entire country, unless the present regulations against interstate commerce in parrots, parrakeets and love birds are strictly enforced.

In spite of the large numbers of cases in recent years and in the epidemics of the past, beginning with the description of the disease by Ritter of a small group of cases in Uster, Switzerland in 1879, very few post mortem reports have been made. However, even from the first description by Eberth[1] of the pathology in Ritter's cases, it is obvious that there is a distinct post mortem picture, primarily that of a unique pneumonic process in the lungs. Eberth described the lungs as showing a gray-red serocroupous hepatization, part lobar and part lobular, in which the exudate was a loose meshwork of fibrin with a few cells. Cloudy swelling of the parenchymatous viscera and a few petechiae on

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

the serous surfaces were also found. Leichtenstern's[4] case, showed a lobar consolidation of both lower lobes and of the dependent portions of the upper lobes. Surfaces made by section showed a soft, smooth, dull, homogeneous appearance, and microscopically a serocellular or cellulofibrinous exudate. In 1930, Siegmund[13] restudied the sections from the case of Leichtenstern and found changes similar to those he describes in his own case of 1929. In the latter a peculiar soft, edematous pneumonia involved both lobes of the left lung and the right lower lobe. The pleura was smooth and the sectioned surface smooth and dark red in color. There was no bronchial or peribronchial involvement. Microscopically the diseased lung showed all stages from a simple edema to complete filling of the alveoli with cells, almost entirely large mononuclears. Fibrin content was slight to moderate. The bronchi were particularly spared. In addition, a moderate acute splenic tumor (280 grams) and cloudy swelling of the liver and kidneys was seen. Microscopically there were large numbers of endothelial cells in the pulp of the spleen, and in the liver the Kupffer cells were increased in number, loosened from the capillary walls, and in some places formed small nests of cells. There was a marked edema of the leptomeninges with increase of histiocytes in the subarachnoid space. The brain was edematous but otherwise not remarkable. An inflammatory hyperplasia was seen in the lymph nodes of the chest, but elsewhere they were not enlarged.

Similar involvement of the lungs was reported by Wilson,[15] Maclachlan et al.,[6] Turnbull,[14] Peterson et al.,[9] and Polayes and Lederer.[10] Wilson found the bronchioles to be practically spared in the process while Machlachlan, Turnbull and Polayes and Lederer found an inflammatory process in their walls, characterized by infiltration with large mononuclear cells, chiefly. Microscopically all the above authors described the consolidation in the lung as being due to an exudate containing varying amounts of fibrin and large mononuclear cells. Polymorphonuclear cells were absent or rare unless secondary infection was present. Of most striking interest were the changes in the alveolar walls in all the recent cases described and stressed by Maclachlan as being

the most distinctive feature of the disease. This consisted of a marked proliferation with enlargement of the cells lining the alveoli, causing the alveolar walls to appear thick. Turnbull's patient showed also hemorrhage in moderate degree into the alveoli and in the most advanced stage thrombosis of capillaries and even of the smaller arteries of the lung. Some alveolar capillaries were thrombosed in Maclachlan's and Polayes' cases.

Oberndorfer's[8] patient who died thirty days after onset, showed large portions of several lobes involved with a hepatization, but obviously a secondary bronchopneumonia was present. Horder and Gow's[3] patient, likewise showed findings characteristic of a secondary infection. The same was true of Russell's[12] patient, who in addition showed aspiration of stomach contents into the respiratory apparatus.

Rivers et al.[11] by insufflation of virus-containing material into the bronchial tree of monkeys produced a bacteria-free lesion corresponding to the findings in human cases, especially characterized by proliferation of alveolar epithelium and proliferation or infiltration of alveolar walls with mononuclear cells, producing a decrease of the alveolar spaces by the thickened walls. The alveoli contained serum, fibrin, and larger mononuclear cells.

The findings reported in organs other than the lungs are not striking. Cloudy swelling of the kidneys and liver, moderate soft swelling of the spleen with proliferation of endothelial cells in the pulp is the rule. Peterson et al., however, found multiple areas of focal necrosis in the liver, many showing polymorphonuclear leucocytic infiltration in the centers and midzones of the lobules. The gastrointestinal tract and the lymphatic system appeared to be relatively spared. Waxy degeneration and hemorrhage into the rectus abdominus muscle was described by Turnbull. Ring hemorrhages in the brain and cord are mentioned by the same author, and as being present in the cord by Polayes and Lederer. Peterson et al. described large ring hemorrhages of various ages in the caudate nucleus and punctate hemorrhages in the corpus callosum, internal capsule and thalamus and floor of the fourth ventricle. Degeneration of myelin in the immediate neighborhood was present.

Although the disease appears to primarily attack the lung in human cases, the lungs are practically spared in birds and in white mice, the latter of which serves as the best test animal for laboratory diagnosis. In birds the most important finding is a large swollen liver and spleen. In mice likewise both organs are enlarged and in addition multiple minute foci of necrosis in the liver are usually found. Only occasionally are necrosis found in bird's livers. Rickettsia-like intracellular organisms called by Lillie "Rickettsia psittaci" or "L.C.L. bodies" by Meyer after the three investigators, Lillie, Coles and Levinthal, who described them about the same time, are best demonstrated in smears from the necrotic areas in the liver or the exudate from the peritoneum of inoculated mice. Smears and sections from human material less frequently show these "minute Gram negative intracellular coccoid and bipolar bacilliform bodies of about 1.2 to 0.3 micra diameter, found in reticulo-endothelial cells, mesothelial cells and large mononuclear cells of the parrot, and in large mononuclear cells in man" (Lillie[5]).

Within the last year two human cases were examined at necropsy by the author. These were the only two who died in a series of ten cases studied clinically by Dr. James B. Luckie, to whom I am indebted for the clinical history. One patient developed an acute exacerbation of an old pyelocystitis and died primarily from this. In addition, there was bilateral secondary confluent broncho-pneumonia which complicated the lung picture so that only one small focus of the original psittacosis remained. The pathology of this was similar to that described in the case below, and further description will not be made. The other patient died of his psittacosis primarily. Material from the lung in both cases, and the liver and spleen in the case below produced typical lesions in mice, the inoculation experiments being made by Dr. Karl Meyer of the Hooper Foundation, San Francisco.

CASE REPORT

Mr. T. J. T., age 57, two weeks before the onset of his illness had purchased two dozen love birds and built an aviary in his yard in order to raise them for commercial purposes. Six of the birds, after developing a diarrhea and a cough,

died shortly after their purchase. On February 10, 1932, the patient abruptly developed a fever, severe headache, and a slight cough. The next day the cough, was more severe, but he could not raise any sputum, nor did he raise sputum at any time until the last three days of his illness. His chest felt "as if it had a tight band around it." His fever and cough persisted, the headache became more severe, and on the fifth night of his illness he became delirious, got out of

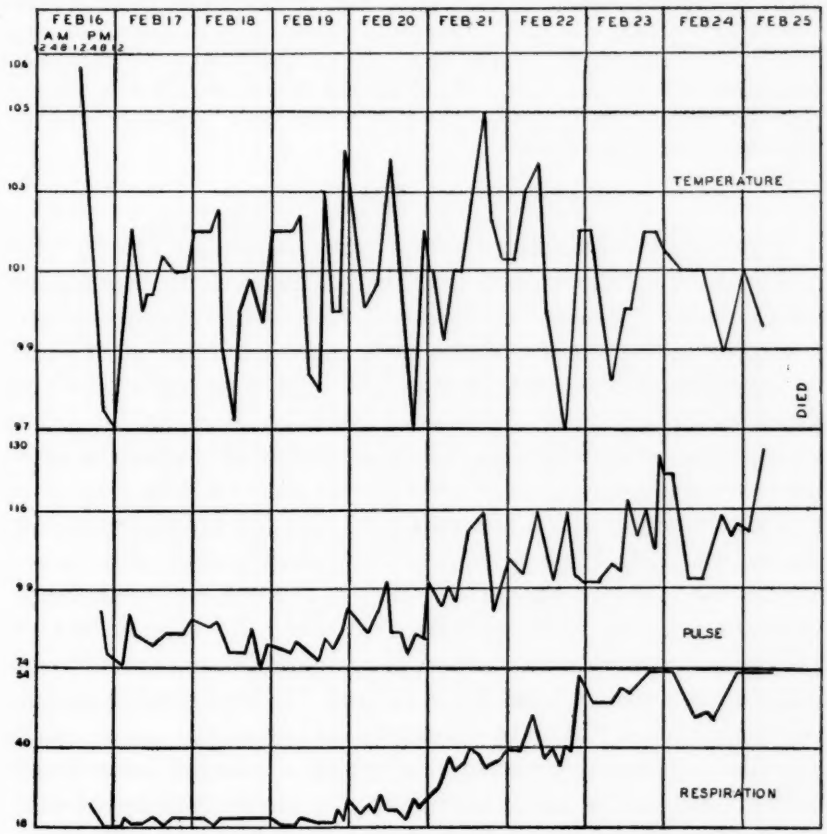


CHART 1. TEMPERATURE, PULSE AND RESPIRATION CURVES OF CASE OF PSITTACOSIS

bed and tried to find his way out of the house. His wife, who became ill two days after her husband, finally restrained him and led him back to bed where both fell exhausted. He was not seen by a physician until two days later, when he was examined by Dr. Luckie. At that time he was semi-delirious, his pulse 100, temperature, rectal 106°, respirations 24. He appeared exhausted, his eyes blood-shot. There was slight redness of his throat. The lymphatic nodes in

his neck were not enlarged. Examination of the chest showed nothing abnormal anteriorly. Posteriorly there was no dullness on percussion, but on auscultation the breath sounds were greatly diminished on the left base, extending upward to the angle of the scapula. Fine dry râles were heard in this area. A few spots resembling rose spots were found on the abdomen.

During the next few days the patient became more delirious, the harrassing cough and fever persisted, dyspnea developed, and râles were found over the entire chest. Death occurred on February 25, fifteen days after the onset of the illness. Chart 1 shows the temperature, pulse and respiration. It will be noted that only in the last few days of the illness was there any marked increase of pulse and respiration, in spite of the high temperature. Necropsy was performed two hours after death.

NECROPSY REPORT SUMMARY

The only external feature of note is a moderate posterior lividity and cyanosis of the nailbed and lips. The head, neck and spinal canal were not dissected because of restrictions. When viewed from below the organs of the neck show nothing of interest. The principal findings are in the chest. Each pleural cavity contains about 500 cc. of clear straw colored fluid, and the pleural surfaces are everywhere smooth and glistening. The left upper lobe is large and is extremely heavy. The anterior rim of its lower half contains air and crepitates normally, but the remaining dependent portion occupying over two-thirds of the lobe is uniformly consolidated, moderately firm to the feel, but yields under pressure without tearing. Surfaces made by section (fig. 1) show a uniformly smooth, almost glassy consolidated parenchyma, gray in color with a faint shade of blue. A considerable amount of fluid with very little blood exudes on pressure. There are no granular portions or areas of hemorrhage. The left lower lobe is only about half the size of the upper, is moderately compressed by the fluid in the chest, and on section shows no consolidation—although considerable blood and some fluid exudes from the boggy sectioned surface.

The right upper lobe, except for the anterior rim and lower border, which crepitate well, resembles the left upper. The mid lobe is unchanged, but the upper half of the right lower lobe posterior is uniformly consolidated, and surfaces made by section are airless, glassy, waterlogged, and gray to gray-pink in color.

The tracheobronchial tree shows a reddened mucosa and the larger branches especially contain a large amount of chunky gray exudate, chiefly fibrin, without mucus or pus. There is a slight soft swelling of the tracheobronchial and hilar lymph nodes.

The pericardial sac contains 100 cc. of clear straw colored fluid and the surfaces are smooth and glistening. The only noteworthy changes in the heart are a diffuse dilatation of all the chambers and a dull, opaque, flabby musculature. Moderate coronary sclerosis is present in the larger trunks.

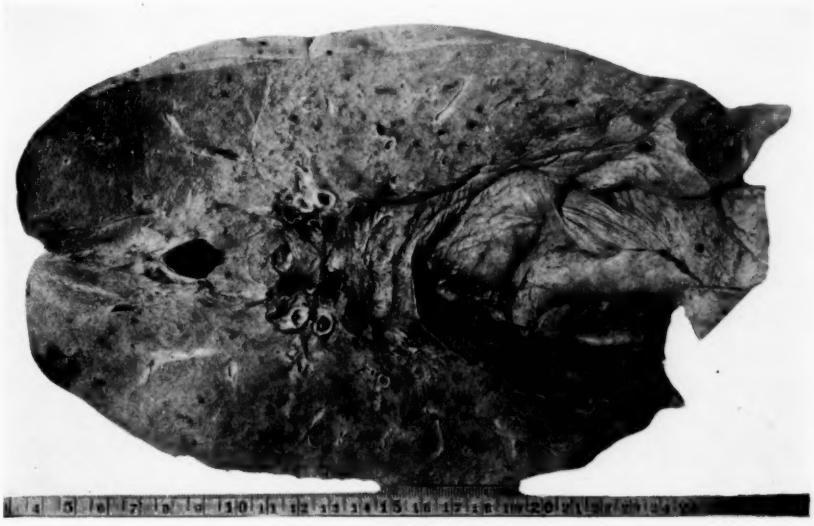


FIG. 1. LEFT UPPER LOBE OF LUNG. SECTIONED FROM APEX TO BASE, SHOWING UNIFORM CONSOLIDATION

There is no fluid in the abdomen. The entire gastrointestinal tract shows a moderate cyanosis of the mucosa. The liver weighs 1900 grams, its edges are rounded, it is firm, purple-chocolate in color, and on section exudes much blood. The lobular markings are plain and the parenchyma is tense and not easily crushed. The gallbladder, ducts, pancreas and adrenals show cyanosis only.

The kidneys are moderately swollen and weigh 325 grams. They show nothing of interest except a diffuse cyanosis. The

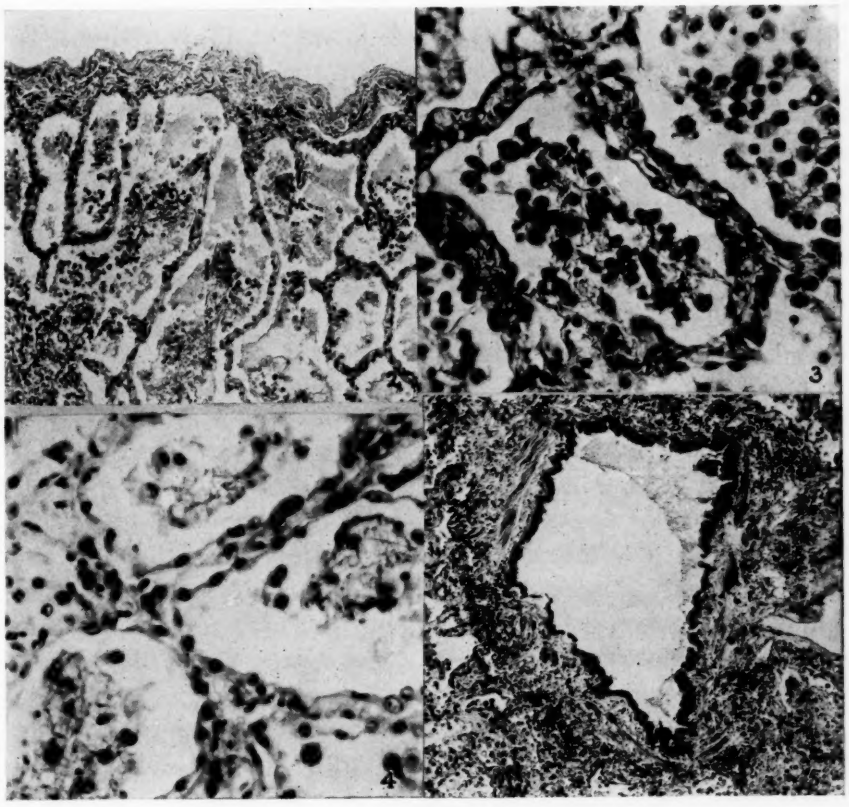
pelves, ureters and bladder likewise show moderate cyanosis of the mucosa. The prostate shows a moderate adenomatous hypertrophy. The testicles and vesicles are free from change.

The spleen is only slightly enlarged and weighs 230 grams. It is somewhat softer than normal, the capsule tense, cuts easily, drains considerable blood, and shows a red-gray, soft pulp from which chiefly blood can be scraped. The Malpighian bodies are just visible.

Moderate arteriosclerosis is present in the aorta and its major branches. The vena cava, iliac and femoral veins are free from thrombi, but fresh thrombi fill the plexus of veins on the left side of the prostate.

HISTOLOGY

Sections through the uniformly consolidated portions of the mid part of the right and left upper lobes show practically similar changes. The alveolar spaces are moderately decreased in size and all are filled with an exudate composed largely of serum with varying amounts of fibrin, which forms a coarse or fine network throughout the entire lumina of the air spaces (fig. 2). Enmeshed in this fibrin are varying numbers of cells, for the most part only moderate numbers, although some alveoli are fairly packed with cells. In all the alveoli the predominating cell is a large mononuclear cell with a single ovoid or bean-shaped nucleus and abundant cytoplasm (fig. 3). Many show phagocytosis of nuclear material, some of red cells, and a few show dust particles. They appear to be typical macrophages. Only rarely is a polymorphonuclear leukocyte seen, although occasional lymphocyte and plasma cell is seen. Erythrocytes are found in small numbers only in a small proportion of the alveoli. Of striking interest is the thickening of the alveolar walls, which appear to be stiff. This is partly due to a congestion in the capillaries, but also largely due to proliferation of histiocytes and to a swelling and prominence of the alveolar epithelium (fig. 4), which shows in many places desquamation of cells into the lumina of the alveoli. The pleura is free from change except for some slight proliferation of histiocytes in its deepest



FIGS. 2 TO 5

FIG. 2. LUNG, INCLUDING PLEURA. SHOWING PLEURA FREE FROM INFLAMMATION. UNDERLYING ALVEOLI FILLED WITH SEROFIBRINOUS, CELL (—) POOR EXUDATE. (LOW POWER)

FIG. 3. LUNG. SHOWING FIBRIN AND LARGE MONONUCLEAR CELLS IN ALVEOLAR EXUDATE. ALVEOLAR WALLS THICK. PROLIFERATION OF ALVEOLAR EPITHELIUM PRESENT. (HIGH POWER)

FIG. 4. LUNG. SHOWING PROLIFERATION OF ALVEOLAR EPITHELIUM AND EXUDATE IN LUMEN. (HIGH POWER)

FIG. 5. LUNG. SHOWING SMALL BRONCHIOLE WITH MINIMAL INFLAMMATORY INVOLVEMENT OF WALL, SURROUNDED BY INVOLVED ALVEOLI. (LOW POWER)

layer, adjoining the involved alveoli (fig. 2). There is no noteworthy hyperemia or cellular infiltration or fibrin deposition. The bronchi in these portions show relatively little involvement (fig. 5). The epithelium is intact in most, and there is an exudate in the lumina composed largely of fibrin and mononuclear cells with a small number of polymorphonuclear leukocytes. There is a slight infiltration of the epithelium with the latter cells in some, and others show a slight infiltration of the subepithelial tissues with polymorphonuclear leukocytes and large mononuclear cells, but some bronchioles are practically entirely spared from inflammatory changes.

Sections taken through the most dependent portions along the lower rims of both the right upper and the right lower lobes show some alveoli which are practically free from exudate, but most of the alveoli contain an exudate which contains only a small amount of fibrin, forming a loose meshwork in which large mononuclear cells are present in moderate numbers, but in addition there are fully as many polymorphonuclear leukocytes. Similar exudate is present in the small bronchi, but here again infiltration of the bronchial walls is not a prominent feature, and many show intact epithelium. The left lower lobe shows the alveoli free from exudate. The alveolar walls are somewhat thickened due primarily to a hyperemia in the capillaries. The alveolar epithelium is fairly prominent in some of the alveoli, and histiocytes are found in the walls in increased numbers, and occasionally about small blood vessels there are fairly numerous histiocytes and young fibroblasts. In a few alveoli, there is evidence of recent organization of the exudate. The bronchi in this portion are not involved. A fairly recent thrombus is found in one of the medium sized pulmonary arterioles and in a few smaller arterioles.

The spleen shows a distinct swelling of the pulp, due partly to congestion and also partly to an increase in the cells, which are chiefly endothelial cells. Many of these are seen in the sinuses and definite endothelial proliferation is found in the walls of the sinuses. Phagocytosis of red cells is seen fairly commonly. A moderate number of plasma cells, lymphocytes, and a few poly-

morphonuclear leukocytes are also found. There is marked encroachment of the pulp on the Malpighian bodies. The arteriolar walls show a moderate hyaline thickening.

The liver shows only a moderate prominence of the Kupffer cells, some of which are swollen and show evidence of phagocytosis of erythrocytes. There is a moderate albuminous degeneration of the cytoplasm of most of the hepatic cells, but very little nuclear changes are noted.

The kidney shows no specific changes. There is a moderate granular degeneration of the tubular epithelium and a few small scars, apparently of vascular origin, just under the capsule, in which atrophied tubules and glomeruli are found. There is slight sclerosis of the large and medium sized vessels. The capillaries are all engorged with blood.

The heart shows some fragmentation of the muscle fibers, and slight edema in the connective tissue stroma, but the fibers stain well.

The adrenal glands show cloudy swelling and congestion and atrophic changes, consistent with the age of the patient.

Smears of the fibrinous plugs found in the bronchi show a few Gram-positive diplococci, apparently staphylococci, most of which are phagocytized, and a few short chains and pairs of Gram-positive cocci. The cells are chiefly polymorphonuclear leukocytes with a moderate number of large mononuclear cells. Giemsa stain shows no bodies recognizable as *Rickettsia psittaci.*

Sections of the lungs, liver and spleen stained by Giemsa are also negative for these organisms.

Gram-Weigert stains of the lung show rare diplococci in the exudate of some of the bronchi, but not in the parenchyma.

Mice injected with material from the lung, liver, and spleen developed typical lesions of psittacosis. (Karl Meyer).

Anatomical diagnosis

Psittacosis, with pneumonic consolidation of the greater portion of both upper and of right lower lobes; subacute splenic tumor; parenchymatous degeneration and dilatation of heart; generalized acute passive congestion; slight bilateral serous thoracic effusion;

slight benign adenomatous hypertrophy of prostate; moderate atherosclerosis of aorta and its larger branches; thrombosis of periprostatic veins.

SUMMARY AND CONCLUSIONS

The principal finding in fatal human cases of psittacosis is a unique pneumonia, involving diffusely the greater part of all of one or more lobes, producing a wet, consolidated parenchyma which is smooth and non-granular on section. The pleura, bronchi, and bronchioles are relatively spared. Histologically the consolidation is found to be due to an exudate consisting of fluid and varying amounts of fibrin, in the meshes of which large numbers of large mononuclear cells are found. Proliferation of the alveolar epithelium and histiocytes in the alveolar walls is a constant finding.

Moderate splenic tumor and congestion and cloudy swelling of the parenchymatous viscera is the rule.

Edema and congestion of the brain and leptomeninges is constant, ring-shaped hemorrhages in the cord and brain have been found in some cases, but thorough neurologic study should be made on all cases, since delirium and psychosis are clinically found in the majority of cases.

Post mortem diagnosis should be confirmed by inoculating white mice with material from the lung, liver, and spleen. Ante mortem diagnosis is usually not possible before the patient recovers or dies but can sometimes be made by inoculation of a series of white mice intraperitoneally with the patient's blood, or better with the sputum.

Since this paper was submitted for publication, a comprehensive report by Dr. R. D. Lillie has been published under the title: "The pathology of psittacosis in animals and the distribution of *Rickettsia psittaci* in the tissues of man and animals." It is published as National Institute of Health Bulletin Number 161. Most of the American cases are discussed and Dr. Lillie has examined the sections of these cases.

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A STUDY OF HYPERPYREXIA REACTION FOLLOWING INTRAVENOUS THERAPY*

HORACE M. BANKS

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Hyperpyrexia associated with chill and fever following the intravenous administration of fluids has been a disturbing factor in this method of treatment. Despite the work done by able investigators along this line, the problem has not been solved. In this investigation it was found that the existence of these fever-chill reactions were much more prevalent in hospital systems throughout the United States than the authorities of each hospital were prone to admit. Various methods have been devised by different institutions to eliminate the occurrence of these manifestations with varying degrees of success. It was discovered that the prevalence of the reaction was looked upon as a factor which should be withheld from the report of the hospital and it was difficult to elicit exact information as to the incidence of occurrence of this reaction in different institutions that were investigated. In some instances rather forceful measures were exerted to suppress any information which might be given out concerning the occurrence of reactions. From various sources, however, it was learned that the hyperpyrexia reaction did exist rather prevalently throughout all sections of the United States, even though much work had been done to educate physicians to the desirability of eliminating this reaction.

In reviewing the literature it is shown conclusively that Seibert[6,7] and Seibert and Mendel[8] solved the problem rather definitely, but the acceptance of this work and the application of these results to practical therapeutic measures has been slow. In view

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

of the definitely concise scientific conclusions based on accurate laboratory studies, it is regretted that an attitude of obstinacy on the part of therapists to accept these facts still exists. The tenacity with which the average clinician adheres to his theory of the cause of intravenous reactions, leaves much to be desired in the hope of further education all along these lines. Experimental procedures controlled perfectly, together with unquestionably correct results appear to be rejected. Around the whole problem seems to be a fog of confusion.

In the pioneer work, Fortune in 1911 reported investigative work which was scarcely heeded. More recently studies were continued by Wechselmann,[10] Tanner,[9] and Seibert and Mendel, but these results have apparently failed to convince the therapist. Few wish to acknowledge that the etiologic factor lies exclusively in the field of bacteriology, yet Mendel and Seibert in their many studies placed it without doubt in the realm of bacteriology.

In 1930 Rademaker[5] gave a very elaborate report of the steps which are necessary to follow in order to eliminate any pyrogenic reaction. In more elaborate detail the Committee of the American Medical Association Council on Pharmacy and Chemistry[3] returned a very detailed and extensive report on the etiology of intravenous reaction, and followed this with recommendations for avoiding the occurrence of the chill and fever. In spite of these authentic recommendations they have not been generally accepted. Other investigators have gone further in the proposal of different types of tests in order to detect the presence of the substance which will here be referred to as a "pyrogenic substance." These tests have in the most part proved inefficient.

The one which was probably the most generally accepted and the most efficient was the one proposed by Carter.[1] This test seemed to carry unlimited promise, but upon extensive application in perplexing situations in which these reactions were occurring in showers, it was found to be totally inadequate. Upon study of the sensitivity of the test it was shown that even if the test as originally proposed was multiplied in sensitivity a hundred fold it would fail to indicate the presence of pyrogen in solutions which were heavily laden with this substance. The failure of

these tests to show the presence of the pyrogenic agent together with the failure of most individuals to follow the recommendations of the Committee of the American Medical Association has resulted in most elaborate proposals of etiologic agents. Essayists have advanced every conceivable factor which enters into the process of manufacturing intravenous solutions. There has been no definite indication as to which factor is most pertinent.

Little[4] has called attention to every factor except the biologic one. In his discussion he has produced no evidence which might identify any one of these enumerated etiologic factors as the specific one. The entire problem presents a rather discouraging situation in any attempt to suggest or produce a satisfactory explanation that would be established by accepted scientific evidence. In view of the above mentioned situation this study was attempted to enhance and reinforce the data which had already been accumulated by previous workers. The study primarily was planned to substantiate or refute the numerous factors, which have been advanced as a causative, and to take into consideration and critically to analyze any test that had been advanced for the study of the detection of pyrogenic substance. The main problem divided itself into the following:

(1) The range of pH of the intravenous solution administered. Are the extreme limits of pH responsible for the reactions?

(2) The rôle the hypertonic and hypotonic salt solution play in the etiology of hyperpyrexia and rigor.

(3) The manner in which particulate matter, such as lint, particles of sulphur from rubber tubing, dust and débris, contribute to these reactions as the causative factor.

(4) The rôle temperature of the solution administered plays in the production of intravenous reaction.

(5) The specific determination of the etiologic agent if possible.

(6) The properties of the specific pyrogenic factor.

(7) The value of proposed tests for the detection of the pyrogenic factor.

METHOD OF PROCEDURE

After careful analysis of all tests the only one thought worthy of consideration was the one devised by Carter. After frequent trials of this test it was found to

be totally inadequate and was soon discarded because it failed to give indication of the presence of massive quantities of pyrogenic substance in the solutions, even when the sensitivity of the test was increased a hundred times. The results were likewise uniformly false and this necessitated a consideration of another test.

In paralleling the work of Seibert and Mendel the normal rabbit was used and it was found in the injection of these animals that whenever the pyrogen was present in appreciable amounts, or even in minute quantities, the animal responded uniformly positively to the presence of this pyrogenic substance. The response of the animal to the intravenous introduction of the pyrogenic substance was prompt, usually within half an hour and continued through a period of three to four hours. When the substance was present in excessive amount the continuation of the reaction lasted as long as four hours. The return to normal was quite abrupt and usually once the temperature had begun to break it returned to normal within a period of an hour or so. The rabbit was selected because of the fact that the temperature range of the rabbit is normally from 101.5° to 104°F. It was noted that temperature conditions under which the animals were kept would also have an effect upon the temperature of the rabbit. When unusually high temperatures prevailed the animal temperature was slightly above that of normal, probably 0.1 to 0.2 degree, and in exceptional instances 0.5 degree. This necessitated keeping the external temperature conditions of the atmosphere constant in housing the rabbits. It was also noted that unnecessarily rough manipulation or exciting influences in the processes of handling the animals stimulated a rise in the animal temperature above that of normal range in a great number of instances. This obviously necessitated manipulation which would eliminate all such factors. With these conditions kept constant the results obtained from innoculation of the rabbit were sufficiently conclusive to indicate that the rabbit as an indicator was probably the most reliable and most dependable test object that could be used as an index for the presence of the pyrogenic factor in intravenous solutions. It was observed through this study that a few drops of water laden with the pyrogenic factor diluted to half a liter by pyrogen free water was sufficient to cause elevations of temperature in the animal to 105°F. and in some instances to 106°F. This solution was equal to a dilution of 1 to 1500, and in this dilution a maximum response was readily obtained. The volume administered was kept a constant factor in all studies. The volume was determined by attempting to parallel the greatest volume given at intravenous therapy following surgical operation and other medical procedures in man. If anything, the object was to exaggerate the conditions which might be produced in the human, in the attempt of therapy when the intravenous solution is given or administration seems indicated.

The first series of animals was given a constant volume of 12 cc. which was estimated to be about one-twelfth of the total available blood of an adult rabbit. It was also estimated that this was approximately in the same ratio of blood volume as the amount given the average human individual who received intra-

venous therapy following operative procedures. Any rise or elevation in temperature above that of the upper limit of normal of the rabbit was indicated as a definite positive response. That is to say a latitude of range between 101.5°F and 104°F was interpreted as normal fluctuations in the rabbit temperature. The variations which were found to occur in the animal's temperature between 101.5°F and 104°F were controlled with normal inoculated animals with known non-pyrogenic solutions. The ranges thus interpreted were so shown that there could not be any positive observations made in regard to the value of the normal fluctuation. After several hundred animals were inoculated it was quite easy to determine the irrelevance of the normal ranges.

TABLE 1

DEGREE OF GROWTH OF *Pseudomonas scissa* AND *Pseudomonas ureae* IN VARIOUS SAMPLES OF WATER

SOURCE OF WATER	DEGREE OF GROWTH
River	Heavy growth
Canal	Heavy growth
Creek	Heavy growth
Cold water main	Moderate growth
Hot water main (softened)	Heavy growth
Well A (city)	No growth
Well B (Riverside)	No growth
Well C (J. L. J.)	No growth
Cistern (J. L. J.)	Slight growth
Well D (M. E. H.)	No growth

The next problem of attack was to point out or to identify from the entire system of intravenous therapy the agent which seemed to be constant throughout all procedures and the one to which importance could be attached as the causative factor of reactions. One factor after another was eliminated as quite obviously excluded from the many factors that might be responsible for the intravenous reaction, until at last the bacteriologic or biologic factor was the one which definitely indicated the source of the fever-chill. The method of investigation was much similar to that used by Seibert and Mendel in the study of water bacteria. All sources of water supply from the city as well as private water supplies were studied from a bacteriologic point of view. In all instances which showed the presence of pyrogen by the rabbit test, these waters also showed the presence of growth of *Pseu-*

domonas scissa and *Pseudomonas ureae*, by cultural methods. In those waters in which cultures gave no growth, none of the members of the chromobacteria group could be identified nor could they be isolated after many attempts. In all of the specimens of water in which a severe pyrogenic reaction was obtained in the animal, a heavy growth of the organisms could be identified, either one or both species being present.

In table 1 the results of the bacteriologic study of various samples of water is shown and it is rather conclusive that water often carries the organisms and that they are not removed by modern treatment of flocculation and precipitation. It can also be seen that the artificial softening process of water as represented by Zeelite process does not remove the organism, nor does it remove the products of bacterial growth. Well water from the deep wells, deeper than 200 feet, is almost constantly free from any of these organisms. It is needless to state that other organisms are obtained from the cultures of water but the group of chromobacteria is entirely absent from deep well water. Cistern water shows irregularly the presence of bacterial strains of this group. Water obtained from various parts of the United States, from the South and the extreme North as well as the extreme East shows the presence of chromobacteria. These samples were obtained as a result of investigations in regard to the probable source of pyrogenic reactions which might come from ampule solutions. In all instances the ampule solutions were entirely blameless and the water from the supply of the hospital always showed the presence of these organisms.

The question was raised as to whether or not organisms of non-pathogenic variety would produce temperature reactions in the rabbit similar to the pyrogenic organisms. Likewise organisms with pathogenic classifications were suggested as possibilities of producing the pyrogenic response. A study of solutions containing killed suspensions of non-pathogenic organisms and pathogenic organisms were given intravenously to animals. From the group of pathogenic organisms *Staphylococcus aureus* was selected; from the group of non-pathogenic organisms common to water, *Bacillus subtilis*. For control a suspension of pyrogenic organisms was

used in the same study. A volume of fluid (pyrogen free water) was used as diluent. A constant volume of 12 cc. of each suspension containing fifty million organisms was the intravenous dose for each animal in this comparative study. The temperature of the animals were recorded for four hours following the injection. It was found that the pyrogenic organisms precipitated an immediate rise in temperature, with a prompt return to normal temperature level after two hours duration. In contrast the pathogens and the non-pathogens used, produced a delayed temperature response which followed an incubation period of an hour to several hours in duration. The return of the temperature to normal was gradual and prolonged over a period of several hours. The animal receiving the pyrogen showed relatively slight intoxication and after two hours of dyspnea and drowsiness was quite active and alert. On the other hand the animals receiving the non-pathogenic and pathogenic organisms were remarkably prostrated and remained so six hours or longer after the pyrogen was active. The specific property of this chromobacterium seems to be that of fever production.

The organisms isolated from the various sources of water supply which possessed the pyrogenic property were easily identified and grew best at room temperature or slightly below room temperature, 25°C. being the maximum temperature at which they grew luxuriently. These bacteria were chromogens and on dextrose agar grew luxuriently with the production of greenish or pale greenish-yellow fluoresence; they were gram negative and actively motile. They were small, plump, rod shaped bodies, about 1 to 2 μ in length, and ends of the bacilli were slightly rounded. These organisms were about 0.75μ in width and in some instances almost simulated cocci. The growth was hazy in outline, particularly of individual colonies, rather serrated when examined under low magnification. The broth culture showed a very turbid growth with a light pellicle formation and this appeared at the end of about forty-eight hours. On potato good growth developed and was elevated with little tendency to spread; a very pale pink color was suggested. In litmus milk the reaction was slightly alkaline, no coagulation or digestion being noted. In gelatine stab there was a good growth but no liquefaction. Nitrates showed a slight reduction at the end of forty-eight hours. Up to this point the two species of organisms could not be separated. At the end of ninety-six hours indol was formed by one species, and not by the other.

When these two species were studied in regard to their fermentation of sugars,

the following results were obtained: *Pseudomonas scissa* produced no acid or gas after seventy-two hours in glucose, lactose, maltose, saccharose, raffinose, manite, inulin, and salicin. In levulose it produced slight acid after twenty-four hours but no gas after seventy-two hours. *Pseudomonas ureae* produced no acid or gas in glucose, levulose, lactose, maltose, raffinose, manite, and inulin

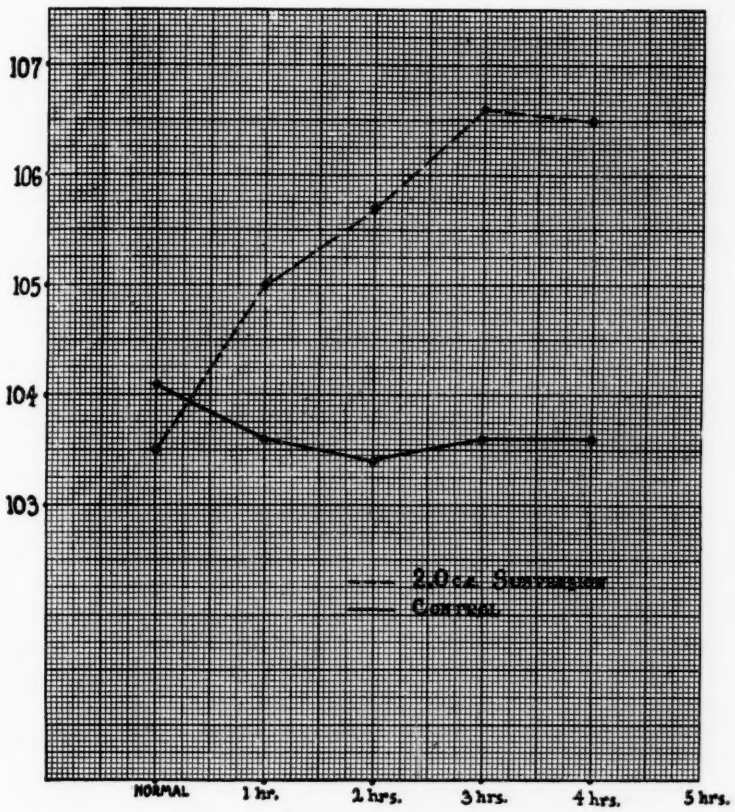

FIG. 1. REACTION FOLLOWING INJECTION OF WATER CONTAINING *Pseudomonas scissa*

but after seventy-two hours produced a very slight acid with no gas in saccharose and salicin. They were classified by following Bergey's Manual of Bacteriology.

Both of these organisms, as well as being isolated from the raw water supply, quite frequently were isolated from intravenous solutions, a part of which had been given a patient and had pro-

duced an intravenous reaction. Studying these organisms together with the ones which we derived from the raw water supply, the two species have been identified and frequently the same species was obtained from different sources. Each of these

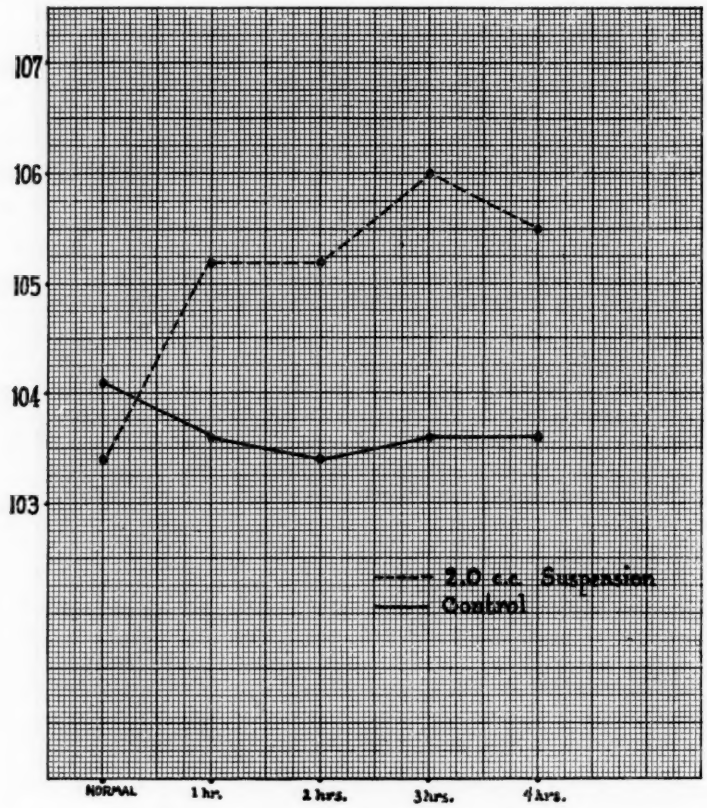

FIG. 2. REACTION FOLLOWING INJECTION OF WATER CONTAINING *Pseudomonas ureae*

organisms produced approximately the same response in rabbits. (See figs. 1 and 2.)

In comparison to the other organisms which were isolated from the water, which were identified as not belonging to the chromobacteria, figure 3 shows that the pyrogenic response is absolutely

specific to these Pseudomonas strains. In the pyrogenic organisms the temperature response is very prompt in the first hour. There is very little or no period of incubation and a rapid return to normal. In the injection of non-pathogens there is a period of

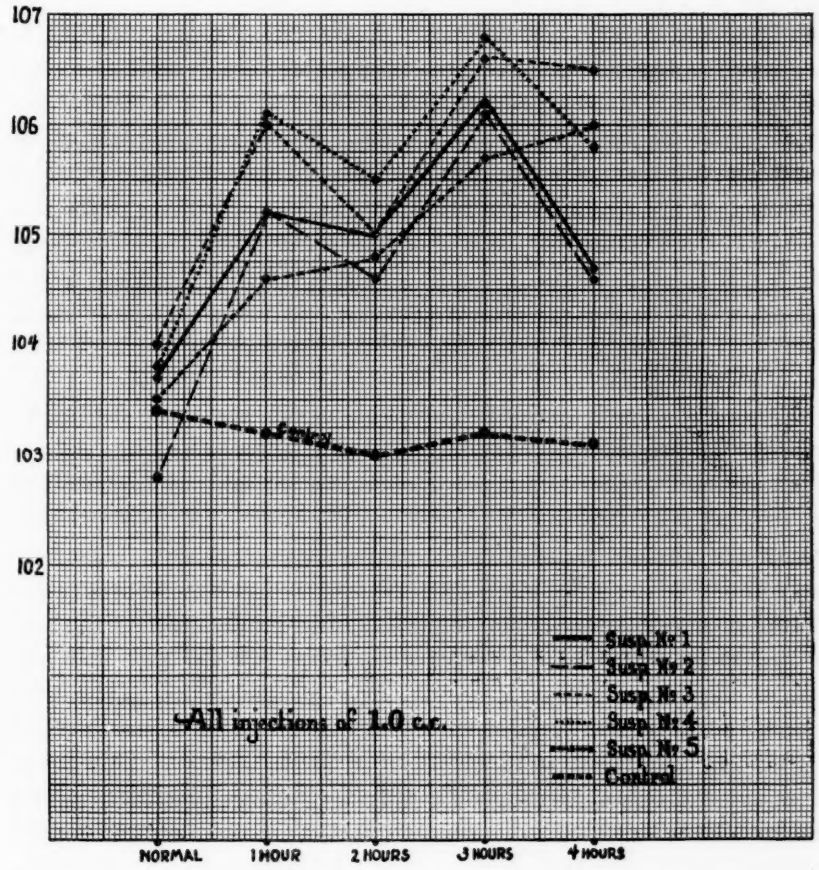

FIG. 3. REACTIONS FOLLOWING INJECTIONS OF PYROGENIC TEST SUSPENSIONS

incubation of an hour or two hours before a temperature response in the animal occurs and then the febrile period runs considerably over an hour or several hours, gradually returning to normal. With the pyrogenic organism the temperature return to normal is very abrupt after a period of several hours.

In the light of these studies it seems justifiable to attribute to these strains of organisms the property of specific pyrogenic activity.

The next procedure was to identify the rôle of other factors in the production of intravenous reaction when the presence of the pyrogen had been quite definitely excluded. In the attempt to accomplish this object pyrogen free water which had given no temperature response in injection of the animal, was used. Sodium chloride was added to this water to make a normal saline solution containing the specific organism. It was then determined whether or not the pyrogen producing substance was a portion of the bacteria themselves or was a substance which was produced as a result of bacterial growth. It was difficult to believe that the growth of organisms occurred in the distilled water, practically devoid of all nutrient materials, which are considered as necessary to bacterial growth.

Using the same constant conditions of the previous studies on rabbits the preparation of killed, bacterial suspensions was diluted with pyrogen free water, so that a suspension of 250 million organisms per cubic centimeter was obtained. Two cubic centimeters of this suspension were given intravenously. The bacterial suspension gave prompt response and a high temperature reaction which lasted over a period of several hours, returning abruptly to normal. An occasional instance was noted in which the animals experienced a severe rigor associated with the temperature rise. Bacterial free filtrates of dextrose broth upon which the organisms were grown were used as material for injection after filtering through Berkefeld filters. The filtrate was controlled as to growth of organisms. In all instances where the controls were negative at a period of forty-eight to ninety-six hours doses of these solutions were given. The uninoculated broth cultures were used as controls. Doses of over 5 cc. of the broth filtrate gave slight temperature reactions of about 0.5° above 104°F. The temperature developed from both types of substances was parallel, the temperature response from the bacterial suspensions was markedly more severe than that obtained by the bacterial filtrates. The substance within the bodies of

the bacteria themselves seems to carry more severe reactions than those substances which are produced extrabacterially. A more recent study has shown in the work on filtrates from the pyrogenic bacteria that the amount of pyrogenic substance generated exobacterially is one twentieth as concentrated in the production of the temperature response as the endobacterial substance. From the above studies it would seem that the pyrogen factor specific to these strains of organisms is an endotoxin rather than an exotoxin, and comes about as a result of the biological composition of the bacteria themselves rather than from the products which are liberated into the media in which they may be present as a result of bacterial growth.

EFFECT OF HEAT WITH AND WITHOUT PRESSURE

A rather interesting development occurred in the process of sterilization of these bacterial suspensions. Inactivation at 58 and 60°C. for one hour were used on some suspensions while on others autoclaving was used, at 20 pounds pressure of steam.

In vaccine work it is generally conceded that the autoclaved preparation is practically valueless and the inactivated preparation of vaccines are considered the most efficacious. It may be seen by figure 4 that in the heated preparation at 58 to 60°C., the temperature response began in about one to one and one-half hours following the injection. In the autoclaved preparation the temperature response began promptly inside of half an hour. The autoclaved product yielded a temperature of 1.5 to 2° higher than the heated product. The autoclaved product was also carefully controlled in regard to the efficiency of the autoclaving and no forms of bacteria could be morphologically identified after this autoclaving process. It is rather interesting to observe in view of the fact that the process which yields the most severe temperature response is based on a process which is used almost universally in sterilization of intravenous solutions. This would seem to indicate that if the solution which is to be used for intravenous work contains any pyrogenic element, the sterilization which is now employed as routine in most hospitals will activate or enhance the possibilities of an intravenous reaction in these

solutions, which otherwise might be eliminated or be considered as latent.

In view of the problem of storage of intravenous solutions in the hospital, the question arose as to whether or not a pyrogen free

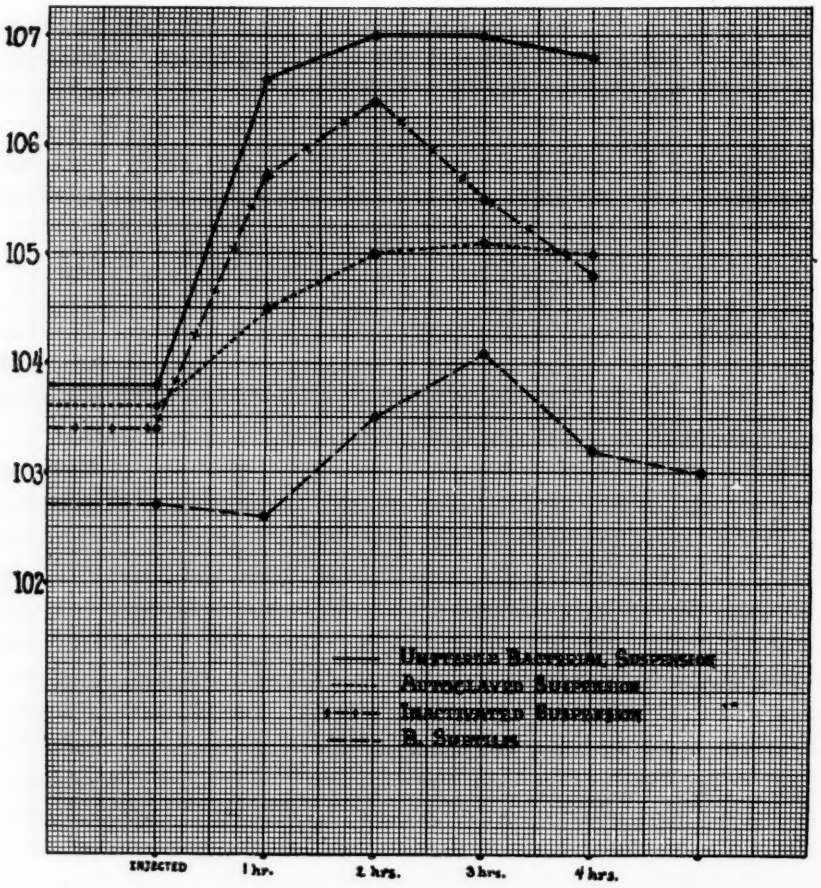

FIG. 4. EFFECT OF TYPES OF HEAT ON PYROGENIC SUBSTANCES

solution might become pyrogen laden upon storage. There also arose the question as to whether or not the pyrogen laden solution increased in its degree of pyrogenic activity upon storage. Under the same conditions relating to the animal experimentation both

pyrogen free water and pyrogen free intravenous solutions were stored. At the same time definitely known pyrogen laden water was stored without any attempt at sterilization or any attempt at careful sealing. At irregular intervals throughout a period of ten months these solutions were injected into animals and observations made as in the first experiment. The results obtained were rather interesting in that the temperature response on the first administration of the contaminated water was maximal and the subsequent injections of the same solution at irregular intervals over a period of ten months gave the same responses. The response to a constant volume showed the same maximal intensity of reaction, that is to say, the first injection of raw water was parallel to and almost exact in all of its details to the reaction which was obtained on the last administration. The pyrogen free saline was stored, together with the water samples found to be pyrogen free. After this long period, on subsequent injections these solutions did not show any pyrogenic activity. The solutions of intravenous type were quite tightly sealed. The water specimens from different sources were not sealed with any more than the usual stoppering.

This study indicates that non-pyrogen bearing water when sealed under average precautions does not become pyrogen laden upon storage. Likewise the same may be said of intravenous solutions which are hermetically sealed; these will remain pyrogen free as long as a period of ten months. It would seem unjustifiable to place the blame for intravenous reactions upon either freshly or stored distilled water when these substances are kept under reasonably careful conditions. It was pointed out by Seibert and Mendel that these strains of organisms grow in distilled water. From such observations as have been obtained it would rather seem that they do not grow in distilled water but are introduced through a process of handling. Once present this substance remains in the water and is difficult to eliminate except through a very efficient distillation process. It may be that freshly distilled water is a medium for growth of these pyrogen producing organisms, but from observations which have been obtained this statement is only true to a certain degree. One qualification

must be fulfilled in order that the growth may take place, that is, that distilled water must be contaminated coming from the still or contaminated in or after collecting.

The pyrogen free water if hermetically sealed remains so indefinitely, stored at any temperature. In view of this fact, properly made intravenous solutions which are known to be pyrogen free will remain so, whether stored one day or one year. The still or the vessels contaminated by the pyrogen are easily freed of this agent by thoroughly washing in distilled water.

SOLUBILITY OF PYROGENIC SUBSTANCE

The next problem in the process of study of this particular factor as applied to use in hospital routine, was in regard to freeing containers which might be contaminated.

If the containers be contaminated what steps are necessary to free the containers of this substance? In other words, is the pyrogenic substance soluble in water, and will mere washing of the contaminated vessels with pyrogen free water eliminate the substance? This is very clearly demonstrated in handling the glassware and also in handling the still. At periodic intervals the still, if not carefully watched or equipped with a spray trap, will bubble over and the pyrogen contaminated substance will be carried into the distilled product. It was found that flasks which contained the pyrogen bearing substance were universally freed of the offending substance by frequent washing with pyrogen free water, say three or four times. It is of little consequence whether or not the pyrogenic laden water or solution has been evaporated upon the surface of the container as it is readily soluble after being dried. Should the still be contaminated by dismantling for repairs or other purposes a run of two hours properly manipulated is sufficient for completely freeing the still of this contamination. In case of discontinuing the operation of the still, it is always safest to run the still for at least one hour before any water is collected for intravenous use. It has been found by frequent examinations that this length of time almost constantly precludes the possibility of any contaminated water. If, however, the water is collected immediately upon beginning

operation of the still it is almost certain, even if the most extreme care is used, to produce a pyrogen laden product. (See fig. 5.)

A study was made to determine the effect of sterilizing water for varying lengths of time in order to learn if pyrogenic substances could be eliminated by this process. It did not appear that ordinary sterilization as routinely practiced would remove the substance. If the finished intravenous solution produces a fever reaction after it has been sterilized for one-half hour at 20 pounds steam pressure after being sealed before and remaining

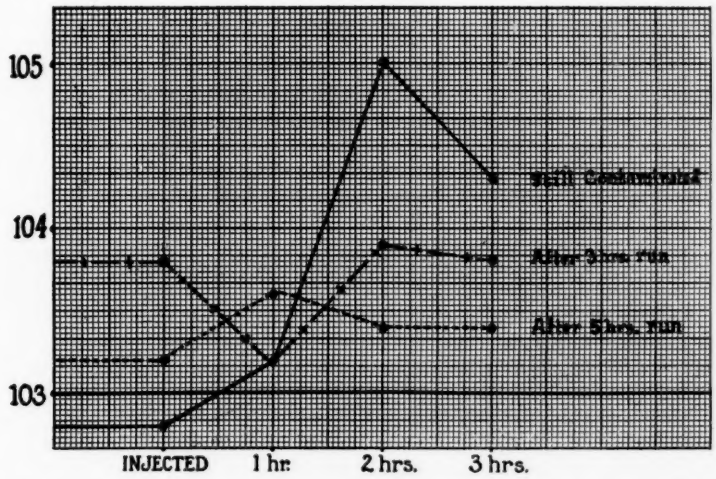

FIG. 5. EFFECT OF PYROGEN CONTAMINATED STILL ON DISTILLED WATER

sealed after sterilization, there must be some factor that is resistant in the fever producing substance to ordinary sterilization ranges. In this study rabbits were used as in the preceding studies and the same volume of fluid was used. The animal which was used as a control was given pyrogen free water of the same volume at the same time. This pyrogen element which was artificially introduced into the pyrogen free controlled water was obtained by using a bacterial suspension of *Pseudomonas scicca* or *ureae* in autoclaved preparation. The seeded solution was injected and produced very prompt rise in temperature in the normal rabbit. The response lasted over a period of time rang-

ing from two to five hours. When one autoclaves this mixture at 20 pounds pressure for one-half hour the response is more prompt than one obtains in the original preparation without autoclaving. At the end of one hour the sterilization of the pyrogenic factor is not

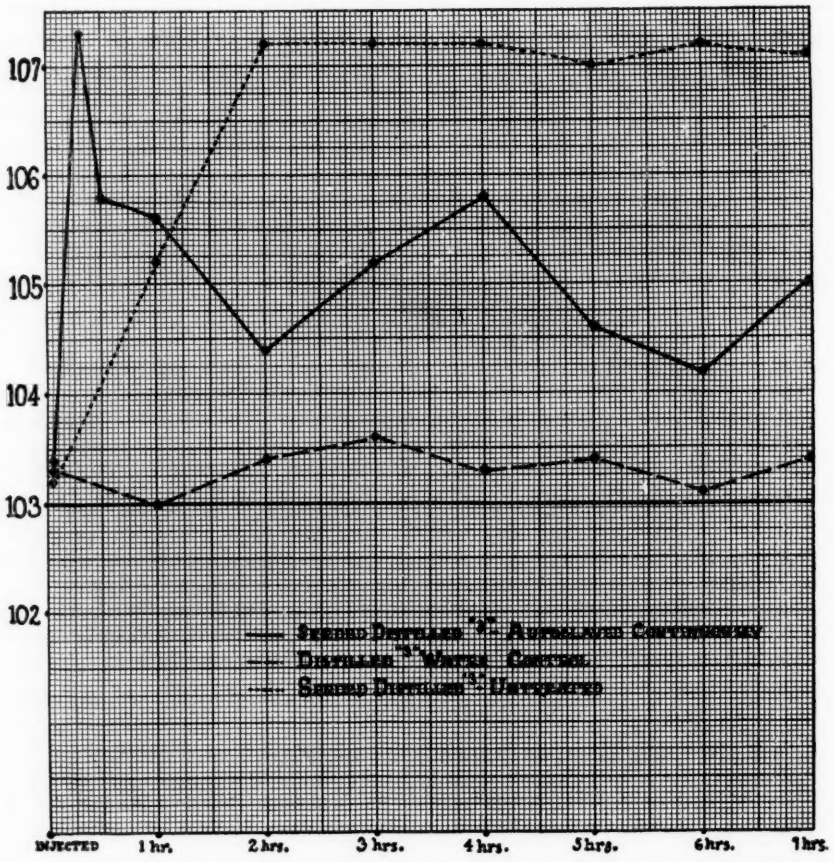

FIG. 6. DISTILLED WATER SEEDED WITH PYROGENIC ORGANISMS AND AT A pH 6.4

decreased. At the end of two hours sterilization there is a slight decrease in the pyrogenic response. At the end of the fourth hour the pyrogen begins to disappear and the febrile reaction is quite distinctly lessened but not until the sixth or the seventh hour does the pyrogenic factor completely disappear and no tempera-

ture response is elicited. Figure 6 seems to convey a justifiable conclusion that ordinary sterilization has no effect in destroying the substance which produces the fever-chill reaction, but seems to enhance or stimulate this activity.

In some institutions investigated it was found that the intravenous solution prepared within the hospital itself showed it to be practically free from pyrogen in all instances. In these institutions it was found they used the same common source of water supply as the majority of the other hospitals. The point of departure in their routine lay in the fact that they used a distillate from the circulating steam, carried at 60 pounds pressure through the mains. This was condensed into water and condensed steam conducted through a distillation process. In the course of circulation through the mains of the high pressure steam system a temperature of 140°C. was maintained throughout the entire system. The pyrogenic factor would be easily broken down at this temperature in a short period of time. It was rarely found that the condensate could be collected before half an hour to an hour's circulation through the system. Thus it seems that from this practical application it is easy to demonstrate that the substance causing the fever-chill reaction is readily destroyed and completely broken down at a temperature of 140°C. in thirty minutes.

HEAT RESISTANCE IN ALKALI MEDIA

In view of the fact that many competent laboratory directors recommended the addition of alkali in sufficient quantity to distilled water to produce a pH of 7.6 when glucose was added to the water, this study was made in order to show the effect of sterilization upon such a mixture. The tests were done with controlled pyrogen free triply distilled water to which was added sufficient alkali to produce a pH 7.6 when glucose was added. This solution was also carried higher in alkali content until the pH was 8.4; and after sterilization this reached 9.2. These alkali solutions in the ranges of pH 8.4 to 9.2 in controlled pyrogen free water were injected intravenously in the animal, and there was no elevation in temperature whatsoever. Pyrogen free solution of pH 8.2 does not give an elevation of temperature. (See fig. 7).

Repeated injections of these solutions confirms the above statement. It was plainly seen that alkali alone added to the distilled water had no influence on the temperature zone. This same degree of alkali was added to the pyrogen innoculated water and it was found that the temperature curve of the animals showed as prompt, as maximal, and as rapid a response as the pyrogen laden distilled water to which no alkali had been added. Consequently

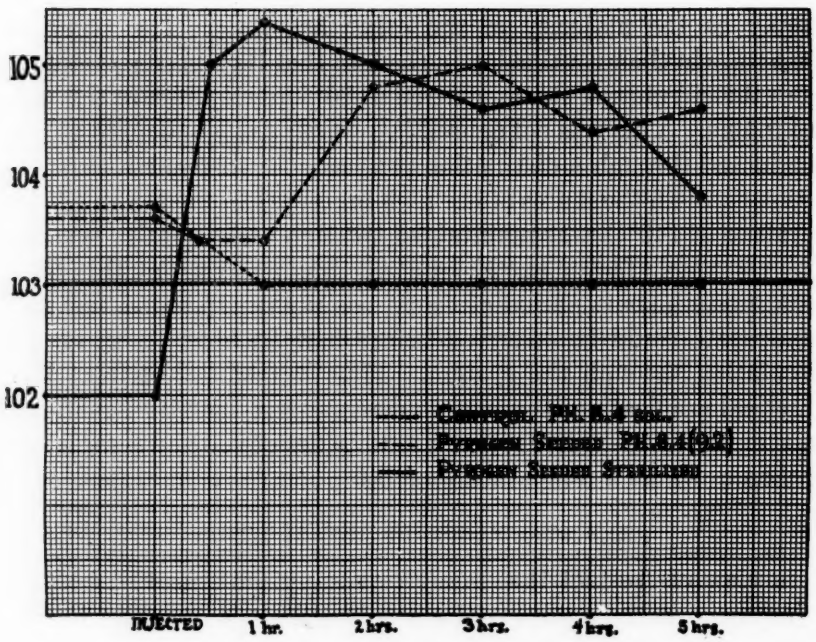

FIG. 7. EFFECT OF ALKALI ON PYROGEN

the mere addition of alkali in the pyrogen laden water had no effect whatsoever. This condition produced artificially by the addition of alkali was added to the influence of sterilization.

It was thought possibly that the heat element might have something to do with the destruction of the pyrogenic factor after the alkali was added or at least caused the pyrogen to disappear more rapidly when subjected to sterilization. In view of this hypothesis the following study was made.

The same constant conditions were produced as in the other studies and the same constant volume was introduced into the circulation of the rabbit with observations every hour for three to four hours. The response was much the same as in the pyrogen contaminated water, except that there was a slight delay in the reaction of the febrile response. The sterilization at 20 pounds showed that in order to have any effect upon the pyrogenic substance four hours continuous sterilization must be employed but the 5th hour of sterilization guarantees complete safety. Thus, alkali itself does not change the character of the fever-chill reaction when added to pyrogen laden distilled water and likewise addition of alkali and sterilization for one hour does not influence the breaking down of this element.

HEAT RESISTANCE IN EXCESSIVELY ACID MEDIA

A contrasting study was made in excessively acid media. In both of these studies it was not attempted to produce a pH in either range that would be intolerable to man if given intravenously, because outside of this range it would be practically impossible to adopt any such measures by the addition of acid or alkali to any solutions that would be able to be utilized in human therapy.

The controlled mixture of pH 3.8, showed on its administration (fig. 8) when made up with pyrogen free water no evidence of fever-chill response. When pyrogen was added the response was quite active, very prompt and considerably increased above the level demonstrated by neutral solutions. With the addition of pyrogen to the pyrogen free solution in a medium of pH 3.8 the fever response was quite prompt. This rise appeared within half an hour after the injection and continued so for three to four hours, then abruptly receded to normal. When this mixture was submitted to 20 pounds pressure of steam for one half hour very little effect was shown upon the maximal response except the fever-chill reaction occurred much more promptly than in the unsterilized mixture. At the end of one hour however, the pyrogen substance showed marked decrease after being subjected to sterilization. The response was very slight as compared to the

original mixture. At the end of two hours the pyrogen was completely destroyed and no febrile response could be elicited from the solution.

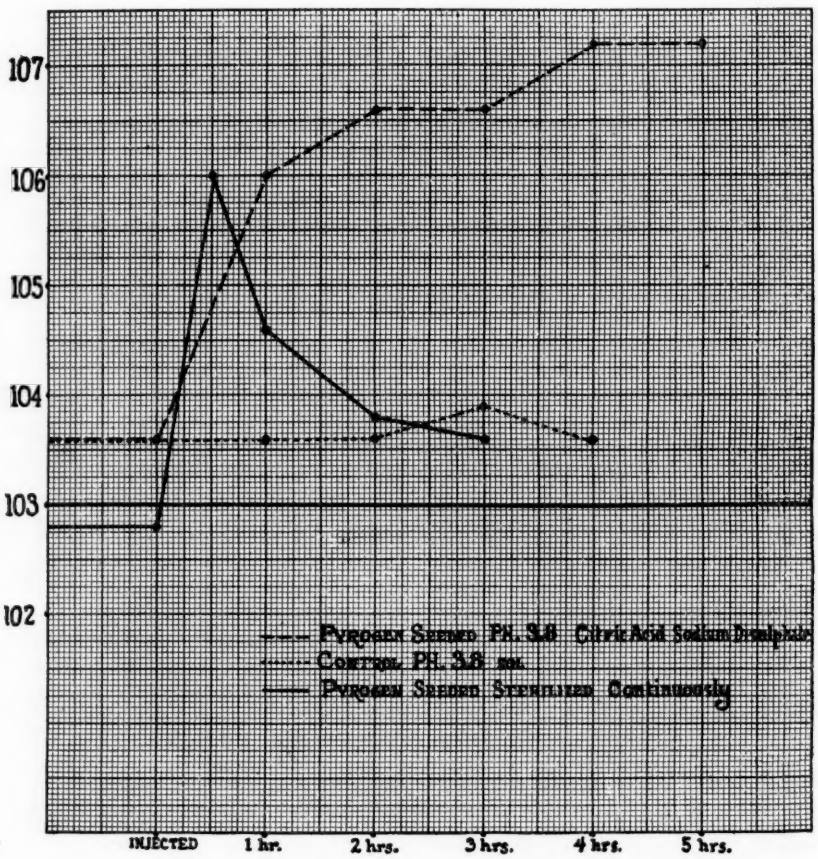

FIG. 8. EFFECT OF ACID ON PYROGEN

HEAT RESISTANCE IN NEUTRAL MEDIA

Up to this point the determination of the resistance of the pyrogen element to heat had been conducted in media which was either of the pH of distilled water or of the excessively acid range or excessively alkaline range. The pH of normal saline which is

usually available from the water of triple distillation results in a pH of 7. Pyrogen free saline was treated with a definite known quantity of bacterial suspension of these chromobacteria. This on being introduced into rabbits in the same volume as that which has been described in the previous studies showed a remarkably prompt response paralleled in a great measure by the same type of temperature reaction that one gets in the excessively acid or in excessively alkaline solution. There is one difference, however, and that is the reaction with the normal saline medium gives a more prolonged temperature response than with either the acid or the alkali. The resistance of the pyrogenic substance in normal saline when exposed to heat, 20 pounds pressure, shows a sluggishness in breaking up. The fever-chill reaction substance is much more active in the solution sterilized for one hour than it is unsterilized, and there is no decrease in the maximum response after the substance has been treated for four hours consecutively at 20 pounds pressure. After the fourth consecutive hour there is an appreciably decrease in the fever chill reaction but it is not marked. There is a gradual decrease in the amount of reaction until the seventh hour is reached and then the fever-chill reaction entirely disappears from the solution.

HEAT RESISTANCE IN DISTILLED WATER

In a similar study the pyrogen-free distilled water was controlled and seeded with the culture of these pyrogenic organisms (fig. 6), and then subjected to sterilization of 20 pounds pressure of steam for each consecutive hour until the substance showed by animal innoculation no response in the fever-chill reaction. In this triply distilled water the reaction is much the same as that which is experienced in the other ranged of pH, regardless of the solute that is placed in the water. The duration of the temperature reaction of the animal is approximately the same as in the neutral solution. When subjected to 20 pounds steam pressure it requires seven hours for it to be completely destroyed. This is quite analogous to the result which is previously obtained in the study of the reaction of the normal saline. Up to four hours continuous steam sterilization of 20 pounds the pyrogenic substance

is not affected, it is quite comparable to the normal saline. At this point there is a perceptible lessening of the intensity of the reaction and from this period of sterilization up to the end of the seventh hour there seems to be very slight change.

Comparison of the graph of the resistance of the pyrogenic factor to steam sterilization shows that in excessively acid media the pyrogenic factor is destroyed in a shorter period of sterilization than any other range of pH. Neutral range shows the least effect on the destruction. The alkaline range shows a definite influence in increasing the rapidity of destruction but the time factor necessary for the destruction of this agent is definitely longer than that of the acid ranges. The theory that the addition of sufficient alkali to readjust the pH of distilled water to neutral after the addition of any pH influencing substances destroys the pyrogen must be considered as erroneous. In the light of these observations it seems justifiable to conclude that the accepted procedures of sterilization of intravenous solutions activate the pyrogen and enhance the chances for reaction, if the water used for intravenous solutions is pyrogen laden. Also the addition of either acid or alkali to pyrogen laden solution shows slight effect on its pyrogen content. The substance is not destroyed or rendered inert by the addition of acid or alkali which seems to increase the severity of reaction. The addition of acid or alkali to a limit tolerated intravenously by living individuals does not cause more rapid dissolution of this factor when exposed to 120°C. for seven hours. This is much too long a sterilization period to be practical and higher temperatures must be used.

In the work done here the results show that 145°C. for one-half hour completely destroys all pyrogenic substances of either the bacterial suspension or bacterial filtrate. In corroboration of this work it has been shown that institutions which utilize condensed steam from their heating plants or the exhaust steam of these plants for distillation have few reactions. This process destroys the pyrogenic factor in the water before being brought to the still. Under this system described, the occurrence of intravenous reaction is inexcusable.

RÔLE OF HYPERTONIC AND HYPOTONIC SALT SOLUTIONS

In further study of the pyrogenic substance the influence of hypertonic and hypotonic salt solutions was investigated. The same factors were duplicated as in previous experiments and the same quantity of solution injected. The hypotonic salt solution made from pyrogen free triply distilled water, was of the strength of 0.4 per cent and the hypertonic salt solution, 1.2 per cent. The animals were injected as in the previous studies and figure 9 shows very slight response in temperature curves, the maximal

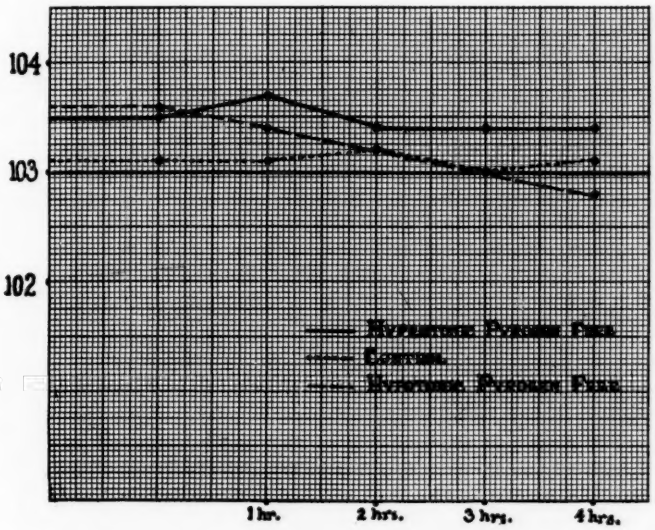

FIG. 9. EFFECT OF HYPERTONIC AND HYPOTONIC SALT SOLUTIONS ON PYROGEN

variation being a few tenths of a degree. The temperature never reached the upper limit of normal in using either solution. The slight increases in temperature were well within the limits of physiologic variations of rabbit temperature. A slight response demonstrated by the administration of these solutions indicated that little credence may be placed in the claim that either hypertonic or hypotonic salt solutions are responsible for the fever-chill reaction following intravenous therapy.

RÔLE OF VOLUME OF FLUID INJECTED AND RATE OF INJECTION

Another factor which has often been given as the cause of intravenous reactions is the volume of the solution which might be administered, regardless of biologic or chemical content. This idea is based on the theory that the volume of the fluid administered at one time might be so massive as to cause disturbance of the water equilibrium between the circulating fluids and the body

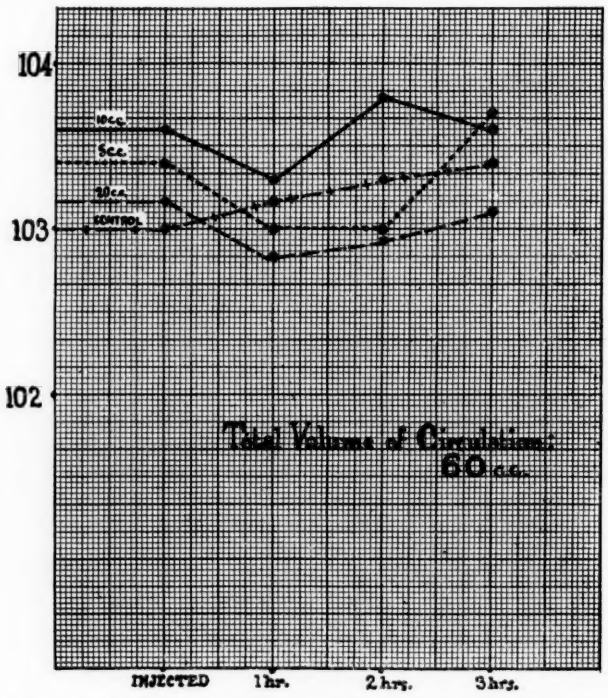

FIG. 10. RELATION OF VOLUME INJECTED TO REACTION

tissues, causing a fever-chill reaction. So popular is this idea among individuals who resort to intravenous therapy that very intricate and elaborate machines have been devised to administer to man large quantities of fluid in small doses over a long period of time. These machines have been so devised as to place a cannula in the veins permanently and to allow through accurate measurement a small amount of fluid to be introduced into the

circulation at regular intervals, thereby eliminating all possibilities of intravenous reaction, the volume itself being the main factor that is considered in the administration of the fluid. No other factor has been taken into consideration in this particular phase of therapy by intravenous route other than the volume. The content of the fluid has not been critically analyzed as to the possibility of what might be causing a reaction.

In the experiments to test this hypothesis the volume of the fluid alone was considered. Varying amounts from a few cubic centimeters to 24 cc. of both pyrogen laden and pyrogen free solutions were introduced. In all the pyrogen laden solutions the temperature response was quite prompt, and the characteristic picture of duration was obtained in all the studies. In the pyrogen free solution regardless of volume, the temperature response was not of abnormal variation, but well in normal limits and infrequently varied not more than one half degree (fig. 10). This variation in temperature is probably ascribed to physiological changes normally present in the individual animal under experimentation. Any volume of pyrogen free fluid itself will not cause a reaction but should the pyrogenic substance be present in the solution, whether the volume be small or large, the response will be unquestionably and unfailingly a fever-chill reaction. During this study the rate of injection likewise was investigated and in all of the 937 animals, the rate of injection was varied at different intervals. In the last several hundred of the series the time of administration was limited to short time, the entire volume being introduced in the animal in the time that is required to rapidly empty the syringe through a 26 gauge needle. In all of these studies it was found that the rate of administration bore little influence to the temperature response of the animal.

IMPORTANCE OF TEMPERATURE OF SOLUTION

In the course of this study attention was called to the rôle that the temperature of the solution administered might play on the temperature of the individual receiving the solution intravenously. In experimental procedure in animals this was reproduced in the most radical extremes that could be encompassed in

the range of solutions administered to the human. Controlled pyrogen free solution was used and was administered to the animal in volumes of 12 cc. The temperature of the solution was then varied from 68°F. to 113°F. Figure 11 shows the influence of the range of temperature of solution.

One animal was selected in which the temperature was at the upper limit of normal. In this instance the temperature of the solution given was the same as the body temperature of the rabbit, that is to say, a temperature of 104°F. In this instance the temperature was elevated 0.2° above the normal range of the animal. The other solutions administered varied in temperature from

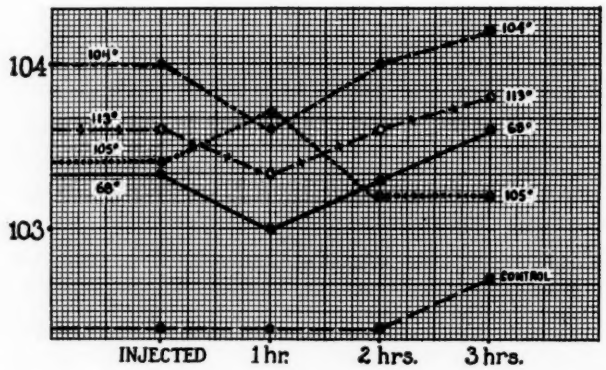

FIG. 11. EFFECT ON REACTIONS OF VARYING THE TEMPERATURE OF SOLUTIONS

68°F. to 113°F. The total range of fluctuation was about one degree. Never was the upper limit of normal of the rabbit's temperature reached at any time during the period of observation. This particular experiment was carried on under conditions in the mid-summer in which the animals were not kept in an excessively cool atmosphere. The same care was taken to provide the atmospheric conditions that would be usually associated with intravenous administration of solutions to the man. In order to deliver the fluid at the temperature which was indicated on the thermometer, the solution was injected through a syringe-needle, and introduced into the animal's circulation as rapidly as possible through a 26 gauge needle.

As to the immediate effects of temperature of the solution administered upon the individual receiving the solution little information or no definite conclusion can be drawn from this experiment. It would seem that the temperature of the solution administered has little influence upon the temperature of the individual receiving the solution. There might be possibility that a solution above body temperature might activate an otherwise latent pyrogen.

THERAPEUTIC APPLICATION

The pyrogenic agent was applied therapeutically through the collaboration of Dr. H. K. Langdon and the clinical application was done jointly with him.

In this study of practical therapeutics in the human individual, these bacterial suspensions were administered to a certain group of patients who had been previously treated by the administration of malarial plasmodia for production of hyperpyrexia. In a few cases this substance was administered to individuals who were rather advanced in central nervous system lues, dementia paralytica and tabes dorsalis, and the results derived were rather surprising (fig. 12). The temperature curve responsed very rapidly in a parallel curve such as is derived from a malarial rigor, lasted from five to six hours, and then receded to normal. A rigor lasting from twenty to thirty minutes was experienced.

The blood count, contrary to that which is derived from a malarial innoculation, during the rigor increased from 90 to 100 per cent. The polymorphonuclear percentage of the leucocytes rose to about 95 per cent.

The condition produced as a result of the administration of this material to paretics is easily controlled. The reaction may be reproduced every day or twice during the day, or whenever the therapist so desires. The patient recovers from the rigor very promptly. There appears to be no dangerous manifestation and temperature ranges from 100.4° to 106°F. are easily obtained. There is no disease to combat after the rigor has passed, and the following twenty-four hours the individual is normal. A rather interesting manifestation which has developed recently in the

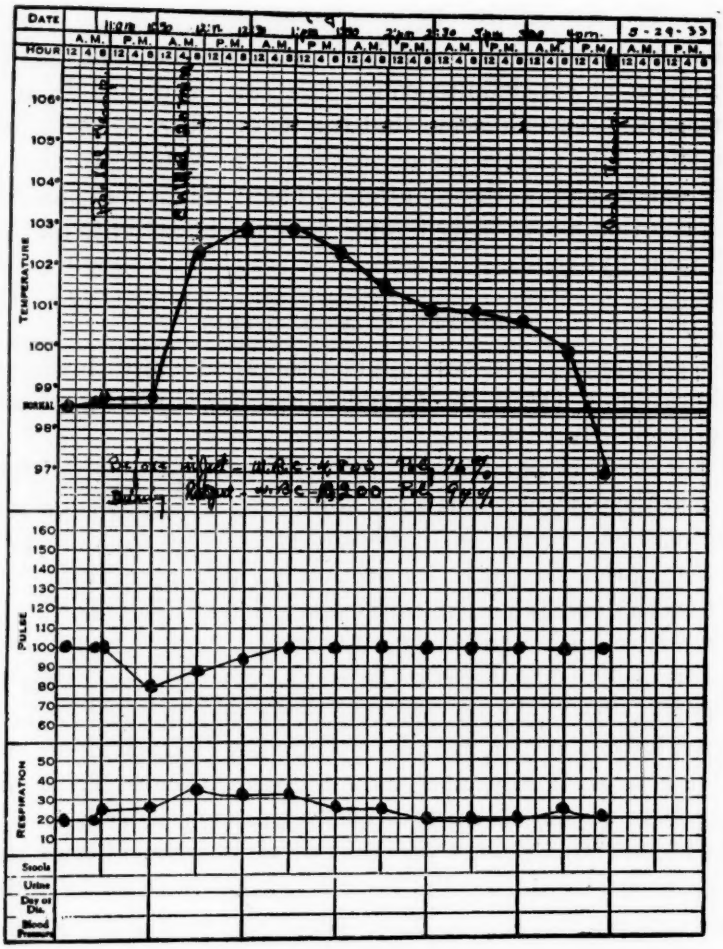

FIG. 12. TEMPERATURE CHART OF A PARETIC RECEIVING PYROGENIC SUBSTANCE FOR THERAPEUTIC EFFECT

Before injection the leukocyte count was 4,800, with 76 per cent polymorphonuclears, after the injection and during the reaction the leukocyte count rose to 10,200, with 94 per cent polymorphonuclears. The temperature was recorded every 30 minutes.

use of this agent is the fact that the hemoglobin and the erythrocyte count mounts steadily during the process of reaction and there is a definite increase in the number of reticulocytes. After

administration of two to three weeks the cerebral manifestations begin to clear up and psychically the patient becomes markedly improved.

The series is too small and the work incomplete so that details of serological changes and laboratory investigations other than that which we have just begun, are not yet available.

DISCUSSION

With the animal studies upon which these investigations were made, it was found that any chemical test as designed for the detection of pyrogen in water or in solutions was totally inadequate. The only test which would unfailingly and constantly show the presence of pyrogen substance in waters and pyrogen contaminated solutions was the use of animals. This test is reliable and without variation. An interesting feature about the use of the pyrogen contaminated solution in intravenous work in animals is that the animal does not become immune to the pyrogen nor is there any evidence that the animal becomes hypersensitive to the pyrogen. In other words, the thermogenic reaction was maximum with all doses of pyrogen whether repeated the same day or several days later. The response in all instances is practically maximum when different sized doses of the pyrogenic substance were given. All doses showed a more gradual reaction in the response, the slightest dose sufficient to give a reaction was 0.03 cc. of a stock solution regardless of what diluted volume was used.

The volume of the fluid injected has little effect upon temperature reaction, provided that the solution is pyrogen free.

The range of alkali and acid solutions from pH 9.4 to 3.8 show little effect in changing the temperature of an animal when large volumes are given, or when small volumes are given, provided that no pyrogen substance is present in the solution.

Particulate matter in dense concentration has practically no effect upon the temperature range of the animal if the water containing the particulate matter has no pyrogen content.

In the study of the effect of hypertonic and hypotonic salt solution upon the fever-chill reaction of the animal, there is no response if the solution contains no pyrogenic substance.

The temperature of solutions administered plays very slight or no influence in effecting the temperature curve of the injected animal. If solutions are used at a temperature above that of the normal animal temperature, it is observed that this temperature will activate an otherwise latent pyrogenic solution. Otherwise if the pyrogen is absent the temperature of the solution has no influence upon the temperature of the animal.

The substance causing the intravenous reaction is specific for that type of reaction. It is derived from the bacterial bodies and products of growth of *Pseudomonas scissa* and *Pseudomonas ureae*. These strains predominate in this reaction. Unless this substance be present in the solution, all other conditions intentionally produced cause no effect upon the temperature curve of the rabbit.

The specific property of this substance, that is, the pyrogenic property, has been shown to be effective for man as well as for rabbits. The pyrogen substance is water soluble and is rapidly eliminated by frequent washings with pyrogen free water. The number of distillations has no advantage in freeing the water of the pyrogenic factor. One distillation is sufficient if the still is equipped with a spray trap. A minimum temperature of 140°C. is necessary for thirty minutes to completely destroy the specific substance. Pyrogenic substances require seven hours sterilization at 20 pounds pressure to completely destroy them. In media of pH 9.4 it requires five hours and a solution whose pH is 3.8 requires three hours at the same pressure in order to completely destroy it.

CONCLUSIONS

The reaction that follows intravenous administration of solutions is an entity characterized by an immediate and prompt rise of temperature in extremely high degree and usually associated with a severe rigor lasting about twenty to thirty minutes. This clinical picture is not to be associated with shock and is entirely separate and distinct from it. Intravenous reaction has a definite, specific, etiologic factor. This factor is the introduction of dead or living products of bacterial growth or cultures of—*Pseudomonas scissa* or *ureae*, into the blood stream of the individual.

These organisms were isolated in pure culture from the offending solution, reinjected into the individual and produced the same clinical picture. It was not possible to recover the organism from the blood stream of the individual suffering from a reaction, even though cultures were made at various times during the rigor.

The pyrogenic substance may be used as a therapeutic agent to produce hyperpyrexia.

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RETICULOCYTE COUNTS IN HEALTHY CHILDREN*

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The reticulocyte count has become firmly established as a valuable indicator of the state of bone marrow activity. It is useful not only as an aid in the diagnosis of anemias, but also as a guide to their effective treatment. It is surprising, therefore, that there is no readily available study of the reticulocyte counts of a sufficiently large series of healthy children of school age to serve as a basis for interpretation of reticulocyte counts in disease.

In the course of a series of studies of normal hematologic standards in adults[1, 2] and children* the optimum conditions for reticulocyte staining were investigated.[3] This article is one of this series and reports the results of reticulocyte counts in children of school age by a method which showed more reticulocytes than the methods in common use.

SUBJECTS

Two hundred and eight children were studied. Of these, 106 were boys and 102 were girls. About ten children of each sex were included for each year between the ages of four and thirteen, inclusive. The age given was that at the last birthday. All had been found healthy at a recent physical examination. They include children from the grade schools, preschool clinic, orphanages, baby homes, and physicians' families. Many were children who were referred to the Doernbecher Hospita for tonsillectomy, but who, on physical examination, showed no indications for this operation. All social classes were included, but, since no significant differences were found in children from the different classes, the sources are not included in the table. All had leukocyte and

* Unpublished data.

differential cell counts and sedimentation rates which showed no striking deviations from the probability curve for normal values in a homogeneous group.* We believe them to be a thoroughly representative group of as nearly healthy children as it is possible to select.

METHOD

Reticulocyte counts were done by a technic which we have previously shown* gives optimum conditions for reticulocyte staining. The technic is briefly as follows:

Equal parts (5 drops) of well mixed oxalated blood and of 1 per cent brilliant cresyl blue in 0.85 per cent sodium chloride solution are mixed in a small test-tube. After one minute or longer a small drop of the mixed material is transferred to a clean slide and a thin smear is made in the usual manner. All reticulocytes in 1000 red cells from consecutive oil immersion fields are counted and the per cent computed. The figures reported in the table are the averages of two or more such counts of 1000 cells each.

Erythrocyte counts were done by the technic previously described.[4] Apparatus with a Bureau of Standards certificate was used throughout. The results reported are the averages of counts made from two or more separate dilutions which agreed within 100,000 cells per cu. mm. of each other.

RESULTS

The results are indicated in table 1. The absolute values per cu. mm. were calculated from the erythrocyte count and the reticulocyte percentage. Note that there are no significant variations for reticulocyte counts at different ages or in the two sexes and that the average ret culocyte percentage of 1.47 for the entire group does not differ significantly from the average of 1.57 found in the study of 160 healthy adults.* The range of 0.4 to 3.8 per cent also corresponds well with the range of 0.5 to 3.8 per cent observed in adults. It is evident that the normal values for reticulocytes recommended for use with adults may also be used for children of this age group. These values are an average of 1.5 per cent and a range of 0.5 to 3.8 per cent.

The absolute counts average 71,473 per cu. mm. and range from 18,640 to 184,800 per cu. mm. They also show no significant variations with age or sex, but as one would expect from the

* Unpublished data.

TABLE 1

Summary of Erythrocyte and Reticulocyte Counts

AGE	SEX	NUMBER OF CASES	ERYTHROCYTES	RETICULOCYTES	
			million	*per cent*	*per cu. mm.*
4	M	11	4.80	1.44	69,188
	F	9	5.00	1.54	77,907
		20	4.89	1.49	73,067
5	M	12	4.93	1.38	66,442
	F	10	4.83	1.80	86,644
		22	4.89	1.57	75,645
6	M	8	5.00	1.64	81,080
	F	12	4.93	1.28	62,161
		20	4.96	1.42	69,728
7	M	11	4.92	1.23	60,305
	F	9	5.02	1.46	73,377
		20	4.96	1.33	66,188
8	M	16	5.16	1.23	62,109
	F	10	5.06	1.60	82,602
		26	5.12	1.37	69,991
9	M	14	5.10	1.40	70,967
	F	10	4.97	1.75	86,524
		24	5.04	1.55	77,449
10	M	8	5.11	1.44	72,976
	F	11	4.95	1.34	65,912
		19	5.02	1.38	68,886
11	M	9	5.24	1.32	69,633
	F	11	5.07	1.83	81,815
		20	5.15	1.50	76,333
12	M	10	5.21	1.47	77,431
	F	10	5.04	1.55	77,325
		20	5.13	1.51	77,378
13	M	7	5.29	1.14	60,404
	F	10	5.13	1.15	58,269
		17	5.20	1.15	59,148
All	M	106	5.02	1.36	68,541
	F	102	5.00	1.58	74,521
		208	5.01	1.47	71,473
Range	M		4.10–6.16	0.5–3.3	25,150–184,800
	F		4.06–5.86	0.4–3.8	18,640–184,680

erythrocyte counts they are lower than the corresponding values for men, 83,160 per cu. mm. with a range of 26,050 to 211,660, and similar to those for women, 72,800 per cu. mm. with a range of 21,400 to 156,800. This variation of the absolute values with the erythrocyte count, while the percentage count remains constant for each age and sex so far studied, is further evidence in favor of reporting reticulocyte counts in per cent rather than in absolute values. There is no apparent correlation between the erythrocyte count and the percentage of the reticulocytes.

COMMENT

These results average distinctly higher than results reported by workers using other methods. Since we have demonstrated that other methods fail to reveal all the reticulocytes actually present, a comparison of our results with such figures does not seem warranted.

It is noteworthy that with our technic no result below 0.4 per cent reticulocytes is found in either healthy adults or children. This permits one to conclude definitely that there is hypoplasia or aplasia of the bone marrow when few or no reticulocytes are found.

SUMMARY

1. Reticulocyte counts in 208 healthy children, about equally distributed as to sex and age between the ages of four and thirteen, inclusive, averaged 1.47 per cent or 71,473 per cu. mm. and ranged from 0.4 to 3.8 per cent or from 18,640 to 184,800 per cu. mm.

2. There were no significant variations in either the absolute or percentage counts for the different sex and age groups.

3. There were no significant variations in the percentage counts from the values previously found for men and women.

4. Reticulocyte counts should be reported in percentage rather than in absolute figures.

5. Normal values for reticulocyte percentage in adults or in children between the ages of four and thirteen, inclusive, are an average of 1.5 per cent with a range of 0.5 to 3.8 per cent, when a

method giving optimum conditions for reticulocyte staining is used.

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CARCINOIDS OF THE APPENDIX*

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Carcinoids of the appendix, because of their clinical behavior and histological structure, form a distinct tumor entity. Oberndorfer[11, 12] was the first to distinguish this type of tumor from true cylindrical cell carcinomata. Although their apparent epithelial nature placed them in the latter category, nevertheless their failure to form local recurrences and to metastasize or produce a destructive growth, together with the fact that they occurred, as a rule, in the early decades of life, placed them in a separate group, to which Oberndorfer gave the name of carcinoid.

Prior to the present century, tumors of the appendix were rarely reported and only in the latter years has attention been focussed upon them. Norment,[10] at the Mayo Clinic, found sixty-seven tumors in approximately 45,000 appendices. At Bellevue Hospital, no case of carcinoid of the appendix is recorded prior to 1916. Between January 1, 1916 and December 31, 1932, carcinoids of the appendix were encountered seven times amongst 9108 appendices. In the postmortem room at Bellevue, carcinoids of the appendix were found twice in 18,700 autopsies. It is probable that more painstaking technic would have revealed a few additional patients. The youngest patient in our series was twenty-three years old; the oldest fifty-four years; the average age was thirty-four and one-half years. Four out of seven patients were males. This is in contradistinction to the experience of most observers who report the lesion predominating in females. Clinically, in so far as a search of the literature reveals, a diagnosis of tumor of the appendix has never been made. In those cases where the lesion is not an accidental finding, a history of abdominal dis-

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

comfort followed by signs of a perforated appendix or peritonitis is usually encountered. The classical symptoms of acute appendicitis, as described by Murphy, Deaver, and others are usually absent.

As to the pathogenesis of carcinoid of the appendix, two major and distinct theories have been advanced. One view, supported chiefly by Ribbert,[13] Aschoff[1] and Oberndorfer, regards them as hematomas, or, as Aschoff designated them, naevus of the mucous membrane. On the other hand, Lubarsch,[7] Miloslavich[9] and others believed these tumors to be a type of true carcinoma arising from the epithelial crypts in the mucosa.

Subsequently, Gosset and Masson[4] on the basis of histological examinations, believed these tumors to represent hyperplasia of special cells containing granules which are chromaffine in nature. Their histological appearance, similar to endocrine tumors, led them to believe they arise from endocrine cells, and hence called them "endocrine tumors of the appendix." Upon further investigation, using special silver stains, Masson[8] noticed that these carcinoid cells were connected with fibers from the plexus of Meissner, and advanced the theory that these growths might be of neurogenic origin, admitting, though, that no proof to support this theory was available.

Danisch,[2] on the basis of painstaking histological work which included a study of the development of the structures comprising the appendix wall in the embryo, proved the theory of Masson and Gosset and showed that these chromaffine cells reduced ammoniacal silver and arose in the primary anlagen of the sympathetic ganglia from undifferentiated embryonal sympathetic elements, and that they can be found in the embryo as early as the fourth month. Using various stains, but especially the Bielschowsky-lithocarmin stain and Sudan III, hematoxylin-eosin, Danisch showed that these tumor cells, rich in lipoidal substance, which is at times noted even in the gross by the yellowish appearance of the tumor, represented a hyperplasia or possibly a regeneration following inflammation, of the argentaffine cells normally found in the intestinal canal (including the appendix) and originally described by Kultschitzky;[6] later in greater detail by

Schmidt.[14] Danisch believed that the elements comprising these tumors are related to the chromaffine tumors of the adrenal and, if so, expected to obtain an adrenalin-like substance from them. Because of lack of fresh material, he was unable to do this.

Hasegawa[5] confirmed the work of Masson in its major part. Forbus,[3] likewise using the silver stains, supported the theory advanced by these workers.

It is the object of this paper merely to confirm the presence of the silver reducing substance in the cells which form the so-called carcinoids of the appendix.

If these studies are correct, then the question arises as to the place of these tumors in oncology. They certainly are not true epithelial tumors; neither are they true neurogenic tumors. They are probably not carcinomata, neuroblastomata or sympathetico-blastomata. They do contain special pigment deposits and Danisch, acting on the suggestion of Pick and Bielschowksy, placed them in the group of pheochromocytomata.

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THE METHOD OF DILUTING ANTIGEN IN RELATION TO THE WASSERMANN REACTION

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Twenty-four years ago Sachs and Rondoni[9] found that for the Wassermann test, alcoholic extracts of tissues diluted by adding salt solution slowly to the extracts with continuous shaking to secure turbid emulsions were more antigenic than when extracts and saline were rapidly mixed to produce opalescent or fairly clear emulsions. They also observed that turbid emulsions were slightly more anticomplementary than opalescent emulsions; that is, the former absorbed slightly more complement themselves than the latter.

In other words these investigators observed that turbid emulsions of alcoholic tissue extracts for the Wassermann test were more antigenic than opalescent emulsions but since then this important factor relating to the manner or method of diluting antigen for the Wassermann test has not received the attention that it deserves.

Browning and McKenzie[2] confirmed these observations and also noted that turbid emulsions of alcoholic lecithin-cholesterin extracts of ox liver were more antigenic than opalescent emulsions but that there were practically no differences in their anticomplementary properties when the complement had been kept for eighteen to twenty-four hours before use.

Noguchi[6] in 1911 recommended the use of an opalescent emulsion of his extract of acetone-insoluble lipoids prepared by quickly mixing extract and salt solution as being "most certain in action" but in 1923[7] advised adding the salt solution drop by drop to the extract to secure the maximum of turbidity and antigenic activity.

Kolmer[4] advised the use of turbid emulsions secured by adding antigen slowly to saline solution and at this time Bronfenbrenner

and Schlesinger[1] found that turbid emulsions of Noguchi's acetone insoluble lipoids were more anticomplementary but opalescent suspensions were more antigenic. Ruediger[8] observed that the degree of turbidity greatly influenced the antigenic sensitiveness of extracts and that for each there seemed to be an optimum turbidity and an optimum dilution for maximum antigenic sensitiveness.

Kolmer and Trist[5] in a study of this subject with various plain and cholesterolized alcoholic extracts arrived at the conclusion that the method of dilution had no apparent influence upon the hemolytic activity of extracts; that turbid emulsions secured by adding antigen slowly to saline or saline slowly to antigen were sometimes but not always slightly more anticomplementary than opalescent emulsions secured by rapid mixing but that *turbid emulsions secured by slowly adding extract to saline with constant shaking were more antigenic than opalescent emulsions secured by rapid mixing.* On the basis of these extensive comparative tests they recommended that antigen for the Kolmer modification of the Wassermann test be prepared by adding the antigen slowly to the saline with constant shaking to secure the maximum of turbidity and the maximum of antigenic sensitiveness.

More recently Wadsworth[10] has advised the rapid mixing of cholesterolized extracts by placing the antigen in a liter flask and the saline in a second flask and pouring the saline on the antigen as rapidly as possible with thorough mixing back and forth several times.

Eagle[3] however recommends a slow mixing to secure the maximum of turbidity by dropping two volumes of saline solution slowly and with shaking into one volume of his alcoholic extract of beef heart sensitized with 0.8 per cent cholesterol and 0.6 per cent sitosterol and after two to five minutes adding 198 volumes of saline to give a 1:200 dilution. Such a dilution has been found more turbid and more sensitive than that prepared by dropping the antigen into the salt solution.

EXPERIMENTAL

This brief review of the literature shows that there is still some difference of opinion as to the best manner of diluting extracts for

the Wassermann test although it appears that the majority of investigators have confirmed the observations of Sachs and Rondoni that turbid emulsions are more sensitive or more antigenic than opalescent suspensions.

This certainly appears to be true of the Kolmer antigen (a cholesterolized and lecithinized alcoholic extract of beef heart) when turbid emulsions secured by slow mixing are compared with opalescent ones prepared by rapid mixing and both titrated at the same time by the Kolmer method. Sometimes there is but little or no difference between the two emulsions of the same extract but in the majority of instances the turbid emulsion, secured by adding antigen drop by drop to saline solution with constant shaking, has proved more antigenic than opalescent emulsions prepared by mixing antigen and saline very rapidly after the manner of diluting antigen for the Kahn precipitation test.

Since the Kolmer antigen is but rarely hemolytic even in a dose of 0.5 cc. of 1:4 dilution we have never been able to see any effect of the manner of dilution upon hemolytic activity.

Furthermore it would appear that there is but little or no apparrent effect upon the anticomplementary activity of this antigen. In the Kolmer complement fixation method the guinea-pig blood is placed in the incubator for an hour or collected about twelve hours previously and kept in a refrigerator before the separation of the serum for complement.

With complement prepared in this manner turbid and opalescent emulsions of antigen gave almost identical anticomplementary units ranging from 0.5 cc. of 1:4 to 1:6 dilutions; but very occasionally the opalescent emulsions, secured by rapid mixing of antigen and saline, have been slightly more anticomplementary (0.5 cc. of 1:8) but never higher than 1:10.

It would appear therefore, that with this particular antigen the manner or method of dilution has practically no effect upon its anticomplementary properties.

There is usually a difference in the antigenic properties of this antigen according to the manner of dilution and in almost all instances the turbid emulsions have been more antigenic than the opalescent ones.

During the past ten years we have made comparative titrations

with twenty different Kolmer antigens. One emulsion of each was prepared by adding 1 cc. of antigen, drop by drop, with constant shaking to 3 cc. of saline to give a 1:4 suspension as recommended in the Kolmer modification of the Wassermann test; these emulsions were quite turbid and may be designated as "slow mixtures." At the same time 1 cc. of antigen was added very rapidly to 3 cc. of saline after the manner of diluting antigen

TABLE 1

ANTIGEN	SLOW MIXTURES (TURBID SUSPENSIONS)	RAPID MIXTURES (OPALESCENT SUSPENSIONS)
1	1:2400	1:1200
2	1:2400	1:1200
3	1:2500	1:2500
4	1:2000	1:2000
5	1:2500	1:2000
6	1:2000	1:2000
7	1:3200	1:2400
8	1:4800	1:4800
9	1:2400	1:2400
10	1:3200	1:4000
11	1:1600	1:2400
12	1:4800	1:2400
13	1:4000	1:3200
14	1:2000	1:2400
15	1:2400	1:2000
16	1:3000	1:2800
17	1:2000	1:2000
18	1:2000	1:1600
19	1:2400	1:2000
20	1:4000	1:4000
Average.....	1:2770	1:2465

for the Kahn precipitation test; these were opalescent and may be designated as "rapid mixtures." Further dilutions of each suspension were made with saline solution in the usual manner.

The antigenic units or the smallest amount giving ++++ reactions in titrations by the Kolmer method was 0.5 cc. of the dilutions listed in table 1.

With these twenty different C.L. extracts (Kolmer) the average antigenic unit of slow or turbid emulsions was 0.5 cc. of 1:2770

as compared with the average unit of 0.5 cc. of 1:2465 of rapid or opalescent suspensions showing the higher antigenic activity of the former. With seven of the extracts the antigenic units of the two suspensions were equal; with ten the turbid or slow mixtures were more antigenic while with three the opalescent or rapid mixtures were more antigenic. But the results have shown that in general terms the Kolmer C.L. extract possesses more antigenic activity when slowly added to saline solution to give turbid suspensions than when rapidly diluted with saline to yield opalescent suspensions.

TABLE 2

ANTIGEN	SLOW MIXTURES (TURBID SUSPENSIONS)	RAPID MIXTURES (OPALESCENT SUSPENSIONS)
1	1:6400	1:6400
2	1:3500	1:2500
3	1:2000	1:2500
4	1:8000	1:8000
5	1:6400	1:2000
6	1:6400	1:2400
7	1:3400	1:2400
8	1:4800	1:4200
9	1:2600	1:2600
10	1:2800	1:3000
11	1:2400	1:2400
12	1:4000	1:4000
Average.....	1:4350	1:3533

During the past two years comparative tests with twelve different Eagle antigens (alcoholic extracts of heart re-enforced with 0.8 per cent cholesterol and 0.6 per cent sitosterol) have shown similar results as shown in table 2.

Turbid emulsions may be also prepared by adding saline solution drop by drop with constant shaking to antigen. Indeed these are sometimes more turbid than when antigen is added drop by drop with shaking to saline solution.

Turbid emulsions prepared by adding 1 cc. of antigen drop by drop with constant shaking to 3 cc. of saline solution have usually proved more antigenic than turbid emulsions prepared by adding

3 cc. of saline in small amounts with constant shaking to 1 cc. of antigen. In one experiment with four Kolmer extracts the antigenic units are indicated in table 3.

Similar results were observed by Kolmer and Trist with plain and cholesterolized extracts tested with primary incubations of one hour in a water bath at 37°C. as well as eighteen hours in a refrigerator at 8°C. so that it would appear that the highest antigenic activity of an extract is obtained by adding the antigen drop by drop with constant shaking to the saline solution.

TABLE 3

ANTIGEN	ANTIGEN TO SALINE	SALINE TO ANTIGEN
1	0.5 cc. of 1:2600	0.5 cc. of 1:1700
2	0.5 cc. of 1:3000	0.5 cc. of 1:2000
3	0.5 cc. of 1:3200	0.5 cc. of 1:3000
4	0.5 cc. of 1:3000	0.5 cc. of 1:2400
Average.....	0.5 cc. of 1:2950	0.5 cc. of 1:2275

TABLE 4

ANTIGEN	BORDET MIXTURE	ANTIGEN TO SALINE	SALINE TO ANTIGEN
1	1:2400	1:2600	1:1700
2	1:2600	1:3000	1:2000
3	1:3000	1:3200	1:2400
4	1:2000	1:2400	1:2000
Average.....	1:2500	1:2800	1:2050

In conclusion brief reference may be made to the Bordet method of diluting antigen which consists of evaporating 1 cc. of extract on a watch crystal and adding 3 cc. of distilled water in amounts of 0.2 cc. while stirring with a glass rod after each addition, higher dilutions being made with saline solution in the usual manner. The resulting emulsions were but slightly turbid and less so than extracts slowly diluted by adding antigen to saline or saline to antigen, drop by drop, with constant shaking. Comparative tests with four Kolmer antigens gave the antigenic units in dose of 0.5 cc. as shown in table 4.

Bordet's method has been particularly recommended for the dilution of extracts of acetone-insoluble lipoids (Noguchi) but it is more troublesome, requires more time and is slightly inferior to the method of diluting by adding antigen drop by drop, with shaking, to saline solution.

CONCLUSIONS

(1) The manner or method of diluting extract for the Wassermann test has a slight but definite influence upon antigenic sensitiveness.

(2) Turbid emulsions of antigens secured by slow or gradual dilution with saline solution are more antigenic than opalescent emulsions prepared by rapid dilution.

(3) Turbid emulsions of antigens prepared by adding extract drop by drop to saline solution, with constant shaking, are more antigenic than turbid emulsions prepared by adding small amounts of saline solution to antigen with constant shaking.

(4) There is no detectable influence in the manner of diluting Kolmer antigen upon the hemolytic properties of this extract.

(5) Turbid emulsions of antigens prepared by slow dilution are sometimes slightly more anticomplementary than opalescent emulsions prepared by rapid dilution.

(6) In the Kolmer modification of the Wassermann test it is recommended that the antigen be diluted by adding it drop by drop to the required amount of saline solution with constant shaking to secure the maximum of turbidity as originally described in this method.

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THE CLINICAL AND PATHOLOGICAL FINDINGS FOLLOWING WARFARE GASSING*

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A study was made of the residual effects of gassing with chlorine, mustard, phosgene, and arsenical compounds, in the case of 486 veterans. Of this number 307 were living and 179 had died. One hundred and thirty-three patients had been gassed with chlorine; 142 with mustard; 139 with phosgene, and seventy-two had been gassed with arsenical compounds.

During the World War the various chemicals used for warfare gassing affected predominantly the respiratory tract. The character of the lesions found immediately after gassing depended upon the particular chemical substance used. Certain of these, as for instance chlorine, affected the upper portion of the respiratory tract; others, like phosgene and the organic arsenical compounds, affected the finer bronchi; mustard gassing was frequently accompanied by a secondary invasion of the respiratory tract with micro-organisms which resulted in acute suppurative conditions of the bronchi or alveoli.

Certain of the warfare chemicals caused traumatic injuries of the eyes. The severity and extent of the eye injuries were dependent upon the chemical used, as well as upon its concentration.

Mustard was the most effective of warfare chemicals in that it

* Abstract of a paper read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933. The study was conducted by Major General Harry L. Gilchrist, formerly Chief of The Chemical Warfare Service, U. S. Army and Dr. Philip B. Matz, Chief of the Research Subdivision, Medical and Hospital Service, Veterans' Administration.

The original studies appeared serially in the January, April, July and October, 1933, numbers of the Medical Bulletin of the Veterans' Administration, also in two brochures issued by the Chemical Warfare Service, U. S. Army.

caused the largest number of casuals. This study disclosed the fact that mustard gassing produced a large number of residual disabilities of various types.

It is believed that chronic bronchitis and emphysema were the most frequent and the predominant residual disabilities noted eight to ten years after gassing with most of the warfare chemicals.

In a number of instances quiescent or arrested pulmonary tuberculosis was reactivated due to the traumatization of the lungs by the warfare chemical.

Both the immediate and the residual effects of warfare gassing noted in this study were similar to acute or chronic infections with micro-organisms. The pathological and clinical findings following gassing were, in a great many instances, affected by complicating superimposed infections with various micro-organisms. This was particularly the case in subjects gassed with mustard.

Emphysema was found most frequently in combination with chronic bronchitis. In certain of the cases emphysema was diagnosed soon after gassing and in other instances it was noted as a residual condition. Emphysema in certain instances was compensatory and was due to extensive atelectasis or areas of congestion. In other instances it was due to an obstruction by an exudate or false membrane in the bronchi or bronchioli, which resulted in an impediment to the outgo of air from the pulmonary aveoli, thus bringing about a distention of the air sacs.

One of the outstanding pathological conditions noted after gassing, especially with phosgene and mustard, was the syndrome known as anoxemia. This condition was characterized by a shortage of oxygen and an increase of carbon dioxide in the blood, due to interference with normal respiration. This led to rapid and shallow breathing and tachycardia. If anoxemia was prolonged, myocardial degeneration was noted. Anoxemia was brought about by either a spasm of the bronchi or bronchioli due to toxic action of the warfare gas, or was the result of an exudate in the bronchi or bronchioli; in either case there was an interference with normal inflow of oxygen and outgo of carbon dioxide, thus an insufficient amount of oxygen was supplied to the tissues.

The effect of the various gases upon the heart was due to one or more of the following factors: (a) An absorption of the gas with resulting tachycardia; (b) a mechanical effect due to obstruction to the pulmonary circulation because of congestion or pulmonary edema, throwing an increased amount of work on the heart; (c) anoxemia resulting in an impoverishment of the blood supply and lessened oxygenation of the heart, with development of myocardial degeneration; (d) a complicating respiratory infection, such as lobar or bronchopneumonia, suppurative bronchitis or bronchiolitis, et cetera, with a deleterious effect upon the heart musculature.

Among the cases studied there were a number who gave a history of residual disabilities due to gassing, from which conditions they subsequently died. The most frequent causes of death among this group were active pulmonary tuberculosis and bronchopneumonia.

EDITORIAL

The Clinical Diagnosis of Brucelliasis in Man

With the advent of clinical appreciation that members of the genus Brucella are infective for man, the problem of clinical diagnosis soon became apparent on account of the varying pathogenicity of this group of bacteria.

Soon after the recognition of this infectious disease in America there arose a great deal of confusion when attempts were made to define what constituted a clear clinical picture. The inability of the bacteriologist to absolutely differentiate between the three more important members of Brucella, namely *melitensis, suis* and *abortus,* no doubt added to this confusion. As time elapsed and the clinical cases were carefully analyzed it soon became clear that *Brucella melitensis* was responsible for the undulating and recurring attacks of fever. *Brucella abortus* was of relatively low virulence often producing subclinical infections and seldom disease of long duration; the fever being of irregular character without the undulations.

During the first wave of enthusiastic interest every serum that agglutinated this group of Brucella was labeled an active infection, but since many of these individuals were symptom free and had questionable clinical histories, certain limits were placed upon the diagnostic significance of the agglutination reaction below 1:160 dilutions. This too was later proved to be of no diagnostic value since it was shown that proved cases of brucelliasis with positive blood cultures had no agglutinins in their blood or agglutinated only in low dilutions. Serologic surveys revealed that serums from about 7 per cent of hospital patients agglutinated bacteria of the Brucella group and from healthy adults about 1 per cent. The agglutinin titers varied from 1:20 to 1:360. Thus it became apparent that the agglutination reaction had a limited diagnostic value.

The introduction of the intracutaneous reaction with *Brucella abortus* was also shown to have a limited value since the test behaves exactly like the tuberculin reaction in that it is only valuable in excluding the Brucella infection when the test is negative. Thus up to 1932 the only absolute evidence of Brucelliasis was the demonstration of the infecting organism in blood cultures or by infecting guinea pigs with blood or pus from a suspected patient.

The recent introduction of the opsonophagic test by Huddleson et al.* offers a new possibility in establishing an absolute clinical diagnosis by laboratory methods without the demonstration of the organism by culture. Huddleson has shown that susceptible individuals have a low opsonophagic power while the immune have a high opsonophagic power. On the other hand the infected individual while exhibiting an opsonophagic reaction of less than the immune and more marked than the susceptible, can be easily differentiated from the susceptible individual by the aid of a positive intracutaneous reaction which is absent in the susceptible person. The writer's preliminary work with a limited number of experiments has so far substantiated Huddleson's report. It is hoped that clinical pathologists will undertake the investigation of this problem and will report individual experiences to the Research Committee for compilation.

—A. S. GIORDANO.

* HUDDLESON, F., JOHNSON, H. W., AND HAMANN, E. E.: A study of the Opsonocytophagic power of the blood of allergic skin reaction in Brucella infection and immunity in man. Am. Jour. Pub. Health, **23**: 917–929. 1933.

NEWS AND NOTICES

The Thirteenth Annual Convention of the American Society of Clinical Pathologists at Cleveland, June 8 to 10, 1934

The programs of the Society have in the past been so superior that it would seem impossible to reach the high standard set at previous meetings. Nevertheless, that it can be done is clearly seen by the tentative program listed below. The Thirteenth Convention promises to be in every way up to standard with any of the past programs. Members who will be forced to miss the meeting will miss not only a profitable but a very enjoyable experience.

The Executive Committee will meet on Thursday evening. There will be a round-table discussion on Friday evening conducted by Dr. P. F. Morse of Detroit following a supper at the Hotel Cleveland. It will deal with the Clinico-Pathologic Conference as well as other interesting questions. The Annual Banquet will be held at 7:30 P.M. Saturday evening. The principal speaker will be Dr. Howard T. Karsner, of Western Reserve University. The business meeting will be held on Sunday evening; the local committee is planning a supper and the meeting to follow immediately after. In general, the program this year will emphasize the subject of malignancy as well as the very pertinent subject of hematology.

Below is a tentative program which, although it will probably be necessary to make certain changes in it, is indicative of the excellent quality of the material which will be presented.

TENTATIVE PROGRAM

Friday, June 8, 1934, 9:00 A.M.

Short Business Session

Scientific Program

Erythroblastic anemias of childhood. A. Yaguda.

Etiology of granulopenia (agranulocytosis)—with particular reference to drugs containing the benzene ring. R. R. Kracke and Francis P. Parker.

Treatment of malignant neutropenia by injection of liver extract. W. B. Martin.
Subleukemic states and leukemoid reactions. A. S. Rubnitz.
Morphologic data in the leukemias. F. J. Heck.
Heterophile antibody reaction in infectious mononucleosis. N. Rosenthal and George Wenckebach.
Blood studies in animals following peptone injection. Harry Goldblatt and E. A. Greenspon.
Direct color photomicrographs by the Finley process on all types of blood cells and abnormal bloods. Russell Haden.
Medico-legal application of isoaggutinins. H. A. Heise.
Further studies on antigen emulsion preparation for the ball test for syphilis. B. S. Kline.
Results obtained with a uniform, stable and lyophile complement. Harry Eagle, Henry Strauss and Rudolph Steiner.

Friday, June 8, 2:00 P.M.

The mechanism of jaundice. N. Elton.
Morphogeographic studies of the thyroid gland, obtained at autopsy. C. A. Hellwig.
Report of an unusual case of siamese twin monstrosity. L. W. Larson.
Oil aspiration pneumonia. Kano Ikeda.
Pyrogen produced hyperpyrexa in treatment of central nervous system lues. H. M. Banks.
Paper on neuropathology. J. W. Kernohan.
Pathological changes resulting from hypo- and hyper-secretion of ductless glands. Anna May Young.
A postmortem analysis of 1000 cases of peritonitis. C. C. Pflaum.
Adrenal diseases in relationship to hypoglycemia and death. J. C. Norris.
Cyanide poisoning: An analysis of cases occurring in New York City since January 1, 1918. A. O. Gettler, and A. V. St. George.

Saturday, June 9, 9:00 A.M.

Paralytic accidents due to anti-rabic inoculations. F. C. Hodges.
Significance of presence of fungi in various locations in the body. Stephen H. Curtis.
Subacute bacterial endocarditis due to diphtheroid bacilli. A. G. Foord and W. J. Stone.
Clinical significance of non-glucose sugars in the blood. R. J. Pickard.
Dextrose tolerance test. W. G. Exton and Anton R. Rose.
Determination of arsenic. A. E. Osterberg.
Diagnostic methods in amebiasis: relative value of stool culture as compared with other methods. C. J. Tripoli and Morris Shushan.

Saturday, June 9, 2:00 P.M.

Early diagnosis of cancer of the stomach. Wm. Carpenter MacCarty.
Malignant hemangioma. B. Markowitz.
Pathogenesis of lung cancer. H. C. Sweany.
Primary carcinoma of the lung. O. A. Brines.
Clinical—pathological aspect of intestinal fibromatosis. Leo F. Bleyer.
Grading of cancer for prognosis. B. Steinberg.
Precancerous lesions of the breast. Max Cutler.
An attempt to define a neoplasm by way of the processes of proliferation, differentiation, and organization. S. P. Reimann.

Registry of Technicians of the American Society of Clinical Pathologists

The first nation wide examination for applicants for certification by the Registry was held last October and proved a pronounced success. The questions were eminently fair judging from the comments of both the examiners as well as those seeking recognition by the Board.

The test took the form of a practical as well as a written examination, the questions having been formulated by a member of the Board of Registry and the examination conducted by a Fellow of the Society or a recognized clinical pathologist in the town nearest the residence of the applicant.

The examiners cheerfully gave of their time and facilities in conducting these tests and conscientiously carried out their duties The first examination brought sixty-six applicants of whom fifty-six successfully passed. Thirty-two examiners participated in the event.

The second examination was held late in April, the results of which are not yet available. Over one hundred and twenty-five aspirants from the United States and Canada filed applications for this examination.

The Board of Registry will hold its annual meeting in Cleveland during the convention of the American Society of Clinical Pathologists. Among the matters to be taken up will be the award of the title of Medical Technologist to those who deserve this designation. Sessions will be devoted to the formulation of a curriculum for training schools and in general to the elevation of the scientific status of the clinical laboratory technician.

Members are urgently requested to attend, and all registered technicians are invited. Non-members will be accorded all privileges except voting. Business sessions are scheduled for Monday, June 11, at 10 a.m., and Wednesday, June 13, at 9 a.m. Scientific meetings will be held Monday afternoon and Tuesday morning. Tuesday afternoon is open for hospital tours, and the annual dinner will be Tuesday evening.

Speakers for the meetings will be:

Dr. Philip Hillkowitz, Chairman, Board of Registry, Denver, Colorado. "The Relation of the Registry of Technicians to the American Society of Clinical Laboratory Technicians."

Dr. B. S. Kline, Mt. Sinai Hospital, Cleveland, Ohio. "The Kline Test for Syphilis."

Dr. Asher Yaguda, Beth Israel Hospital, Newark, N. J. "The Requirements and a Tentative Curriculum of Approved Schools for Technicians."

Dr. W. D. Stovall, Director of State Laboratories, Wisconsin Board of Health. "Molds and Fungi and How They Effect the Technician."

There will also be scientific papers read by members of the society.

The Convention headquarters will be the Carter Hotel, Prospect Avenue and East Ninth Street, Cleveland, Ohio. Send reservations for annual dinner to Mrs. Christine C. Seguin, 8056 Lincoln Avenue, Niles Center, Ill. Tickets for the dinner will be sold in Cleveland for $2.00.

Word has reached the Registry of the formation of the Texas Society of Clinical Laboratory Technicians. An enthusiastic meeting was held in San Antonio, December 9, 1933. Constitution and By-Laws were adopted. Committees were appointed and the following officers elected:

President, Mr. H. A. Bardwell, San Antonio.
First Vice President, Mrs. Pauline S. Dimmitt, Sherman.
Second Vice President, Miss Elizabeth Pickett, Dallas.
Secretary, Mrs. Ida F. Levinson, Houston.
Treasurer, Mr. George T. Thomas, Beaumont.

At the banquet, the following physicians addressed the meeting: Dr. T. S. Roach, Dr. B. F. Stout and Dr. J. H. Moore.

The next meeting will be held in Dallas, Texas, during the week of the State Fair.

BOOK REVIEWS

Aids to Pathological Technique. By David H. Haler. Pp. x + 187. Baltimore, William Wood and Co., 1933. $1.50.

This pocket-sized manual is intended for students and other laboratory workers and especially for those about to take examinations. The author has selected only one method for each test and has avoided any but the briefest discussions. An idea of the content of the manual may be gained from the section headings: Bacteriology, Hematology, Cytology and Parasitology, Biochemical Methods, Preparation of Media et cetera and Stains and other Formulas. The methods given frequently are those known in England but not used in the United States and the fact that inoculation of guinea pigs for tuberculosis is omitted because they are done only in laboratories licensed by the "Home Office" will not help the rest of the world. A large number of colloquial expressions and references to old world apparatus appear. The nomenclature in Bacteriology is badly mixed with old and new names. Some examples of careless writing occur, as for example, reference to cultural methods for "Cysticercus hooklets of various worms;" abbreviations in a table on page forty-three are not apparent; Taenias are considered as a class on page ninety-four, as separate from other cestodes, and tapeworm infestations are supposed to be diagnosed by finding the worm or its scolex, no mention being made of the ova. The advice to draw blood from the ear by puncturing with a glass capillary is certainly dangerous and uncalled for. The omission of flocculation tests for syphilis because the Ministry of Health requires the Wassermann well indicates the breadth of the book.

Text-Book of Pathology. By Robert Muir. 3rd Ed. Pp. vii + 957. Baltimore, William Wood and Co., 1933. $10.00.

This edition contains many important changes in the text made necessary by the advance in knowledge of the subject and some

parts have been rewritten. A number of additional illustrations to the already numerous ones have been included. They are all of excellent quality and most of the photographs are from specimens in the Museum of the Western Infirmary, Glasgow. While the presentation of the subject is from the standpoint of a teacher of pathology and hence is intended as a text, the author emphasizes the relation to clinical medicine and surgery. The book is divided along orthodox lines and deals with general and special pathology. While the text is clearly written and the presentation of the subject good, a distinct criticism is the lack of references to original articles. The student should be encouraged to read outside of the text by giving him numerous references. For example, if this had been done he might discover that the oncosphere of *Diphyllobothrium latum* has a very definite way of reaching a fresh water fish and does not get there "in some way" as indicated on page 454. Also it was Gosset and Masson who described the silver reaction in carcinoids of the appendix and who spoke of "endocrine adenomas" and not Masson alone. Masson, eight years later put forward the theory of the origin of certain cells in these tumors from nervous tissue, a fact omitted in the text. The book contains an elaborate index.

Histology. By S. Ramón-Cajal, Revised by J. F. Tello-Múñoz, Translated from the Tenth Spanish Edition by M. Fernán-Núñez. Pp. xiv + 738. Baltimore, William Wood and Co., 1933. $8.00.

American students will receive with pleasure this English edition of the most famous Spanish text in anatomy from the master histologist of the present time. Because Cajal's major works have been published in his native tongue most American students have not known of his investigations at first hand. This text is especially complete and beautifully illustrated with 535 figures, most of which are original or from the author's pupils. The first chapters are concerned with cytology and early embryology. Then follow chapters dealing with the tissue systems. These are followed by consideration of the histology of the organ systems and finally by chapters dealing with methods which are especially

valuable. Naturally the outstanding chapters deal with the nervous system and here one finds a treatise of about 200 pages and in addition twenty pages on neurological technic. These chapters give a complete record of the great master's contributions to the subject and make available quickly, material otherwise almost impossible to obtain. References throughout the book are limited to Spanish investigations. The book is as thorough and comprehensive as a text should be and may be accepted as entirely authoritative.